MILLER

Studies in the
Artificial Digestion of Meat

Chemistry

A. M.

1907

STUDIES

IN THE

ARTIFICIAL DIGESTION OF MEAT

BY

DONALD S. MILLER, B.S., 1906

THESIS

FOR THE

DEGREE OF MASTER OF ARTS

IN THE

GRADUATE SCHOOL

OF THE

UNIVERSITY OF ILLINOIS

PRESENTED JUNE, 1907

UNIVERSITY OF ILLINOIS

May 31 1907

THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Donald S. Miller, B.S., 1906

ENTITLED Studies of the Artificial Digestion of Meat

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

DEGREE OF Master of Arts

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Experiments on digestion are of two kinds, natural and artificial. Both methods have been used for many years to determine the digestibility of food. The first experiments of course were by the natural method, and many devices were invented for watching the progress of digestion, and determining its results. Experimenting is still being done along this line for many purposes. Artificial digestion experiments were first contrived to take the place of natural digestion experiments. In the development of methods for this work however, many difficulties had to be overcome. Objections have been raised that the artificial digestion does not represent the same conditions as natural digestion, and therefore the results obtained by the two methods are not comparable. The differences in the two methods are summarized by Lea[1] as follows:-- In artificial digestion there is no motion of the contents, no continuous additions of fresh fluid and no removal of digestive products. Sailor and Farr[2] list the factors in natural digestion not found in artificial digestion as follows:--

1. Indigestion of food, water, and saliva.
2. Secretion of gastric juice.
3. Secretion of mucus.
4. Osmosis.
5. Discharge of contents through pylorus.

6. Frequent regurgitation of the individual substance.

This however does not destroy the value of the artificial experiments. It has often been shown that the total digestibility by the two methods is about the same. The artificial method has a distinct advantage in the following ways. It is less cumbersome, it has fewer sources of experimental error, the method can be modified to suit a variety of purposes, and it is a convenient method of determining the rate of digestion.

The last two factors are of especial importance. If it is desired to determine the effect of different strengths of acid or pepsin solution, or to stop the fermentation before complete digestion for any purpose, it can be done much more easily than with natural digestion experiments. This point has been emphasized particularly by H. S. Grindley and T. Mojonnier, in their work in this laboratory, on the comparative digestibility of raw and cooked meats.

History of Artificial Digestion of Foods.

The history of artificial digestion really begins with the work of Stutzer.[3] In 1880 he proposed a method which he thought would be of great value. He extracted the fat from a small sample of food, and digested the remainder for 24 hours in an acid pepsin solution. Then he filtered off the residue

to the Patent Office, and the Patent Office has granted the patent.

The patent has been granted to the inventor, and the inventor has the right to use the invention for his own benefit

and treated it with an alkaline solution of pancreatic extract. Finally he determined the N in the undissolved portion. Such treatment, he thought, represented approximately what took place in the stomach and duodenum.

Niebling[4] modified this method by heating the contents of the flask to boiling for fifteen minutes after digestion with the acid pepsin for 24 hours, and neutralizing with Na_2CO_3. Then the alkaline pancreatic solution was added without filtering and the digestion continued for 6 hours. The N in the residue was determined.

Wilson[5] filtered off the residue from the pepsin digestion, and treated it for 12 hours with a pancreas solution made by dissolving 1 1/2 gm. Merck's pure pancreatin and 3 grams Na_2CO_3 in 1 l. of H_2O. The N in the residue was determined.

Pfeiffer[6] compared natural with artificial digestion, and concluded that the treatment with the pancreatic solution in the latter method is unnecessary.

Kühne[7] found that 48 hours was necessary for complete digestion by the acid pepsin.

Kühne, Bornstein, and Zietstorff[8] obtained good results without removing the fat from the food before digestion.

Beaumont[9] compared the rate of natural and artificial digestion, and found that the former required only from 1/4 to

1/2 as much time for complete digestion as the latter.

Jessen[10] was among the earliest to work upon the total digestibility of raw and cooked meats. His method is outlined as follows by Dr. H. S. Grindley and T. Mojonnier. Two hundred and fifty grams of beef were freed as completely as possible from sinew, fat, gristle, and bone; and similar portions of it were half boiled, well boiled, half roasted, and well roasted. The cooked meats were then partially dried; and a sample of each, and also of the raw meat, weighing 25 grams were treated with 400 c. c. of an acid pepsin solution containing in some cases 1 gram and in others two grams of pepsin per liter of either 0.1 or 0.2 per cent HCl. The digestion was continued for 24 hours, with frequent stirring, at a temperature of 37° C. The insoluble residue was then removed by filtration, dried at 100-110° C. for 2-5 hours, and weighed. From these weights, and the weights of the original samples, the proportions digested were computed.

He concluded that raw meat was more easily digested than cooked meat.

Chittenden and Cummins[11] also worked on the relative ease of digestion of raw and cooked meats. Their results agree with those of Jessen.

Popoff[12] conducted experiments with both raw and cooked meats to see which digested the most easily. He digested each kind

of meat for periods of 3, 4, and 5 hours and arrested digestion by neutralizing with $CaCO_3$. His results corroborated those of Jessen in regard to the digestibility of raw and cooked meats.

Stutzer,[13] again in 1892, reported some results on artificial digestion. His problem, like that of some of the others, was to determine the relative digestibility of raw and cooked meats, and like them he concluded that raw meat was more easily digested.

In the digestion experiments so far, reported here, it is evident that these investigators worked for three things. 1. To devise a method of artificial digestion which would give results similar to natural digestion experiments. 2. To determine the total amount of food digested under certain conditions, and 3. (in some instances) to determine the relative digestibility of raw and cooked meats.

With the exception of Popoff and Stutzer, they did not give any information as to the rate of digestion; and none of them attempted to get much data concerning the effect of different methods of cooking upon the ease of digestion.

Experiments along this line have recently been conducted in this laboratory by H. S. Grindley and T. Mojonnier.[14] They worked almost exclusively upon meat, and obtained a large number of results showing the relative ease of digestion of raw meat,

and meat cooked in different ways. As it was necessary, in their work, to lay special stress upon the rate of digestion, the artificial method was far superior to the natural. The methods used by previous investigators, however, were not well adapted to the new line of work. A new method was then adopted which may be outlined as follows. The meat to be tested was ground thoroughly by passing three times through a sausage mill and mixed with the hands. It was then dried and powdered, and portions of from 0.8 to 1.2 gram were weighed out into 2 Jena beakers for digestion. (In later experiments this was modified by substituting a sample of from 2.00 to 2.5 grams of fresh meat for dried meat.) To the contents of each beaker were added 100 c. c. of an acid pepsin solution containing 2.5 grams of pepsin per liter of 0.33 per cent HCl solution (later the strength was changed to 1.25 gram to a liter of 0.33 per cent HCl solution). The beakers were then placed in a water bath, kept at 40° C., and digested for the required length of time. After digestion they were removed and filtered. (Another modification was made in later work by adding 10 c. c. of 40 per cent formalin to stop further digestion.) Filtering was accomplished by pouring the contents of the beakers into a funnel containing a corrugated hardened filter paper. The filtrate from this filter was refiltered by passing through a second filter fixed

in a similar way, just beneath the first one. The residueson the two filter papers were washed and the N determined by the Kjeldahl process. The per cent of nitrogen undigested could then be calculated by dividing the N in the residues by the total N in the original sample. To get the per cent digested this value was subtracted from 100.

Object of the Present Study.

It is the object of the experimental part of the present study to try to improve upon the method just outlined; so as to detect small differences in digestibility; and to shorten the operation sufficiently to make it useful for an extended series of experiments. The specific purpose for which a new method is desired is to determine the relative end of digestion of different kinds and different cuts of raw meat.

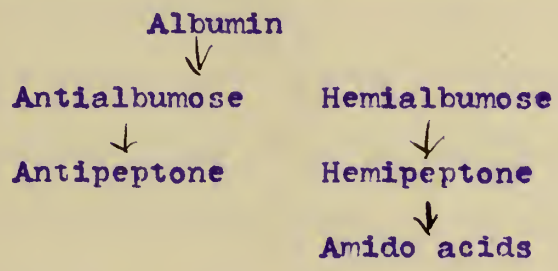
The Chemistry of Artificial Digestion.

At this point it seems advisable to outline briefly the action of the gastric juice, or the acid pepsin solution upon the proteids of flesh.

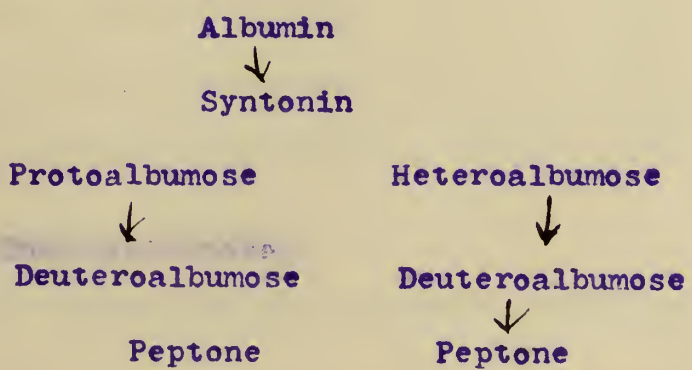
Flesh probably contains a large number of different proteid bodies. Each has its own characteristic properties,

but the action of pepsin solution is probably of the same nature on all of them. Pepsin itself is an unorganized ferment, secreted in acid solution by the mucus membrane of some animals, to produce fermentation of food. When the proteids of flesh come in contact with the pepsin in presence of HCl solution, a charge is produced called proteolysis. 'This[15] consists in the breaking up of the very complex albumen molecule into smaller ones, the size and constitution of some of which are known.' The products thus formed have been studied both qualitatively and quantitatively by numerous investigators. Plimmer[16] outlines by diagram the theories of greatest moment.

Thus Kuhne thought the action went as follows.



Chittenden and Neumeister obtained results which may be represented thus.



Frankel, Langstein, Salkowsky, and others have also worked upon the digestibility of different kinds of proteid bodies. Each separated compounds or groups of compounds at different stages in the digestive process and all agree pretty well as to the properties of the compounds formed. As a result we are safe in accepting the diagram of Chittenden as representing what takes place in the gastric digestion of meat.

This diagram of course does not represent all that takes place in gastric digestion. The action goes far beyond the peptone stage and gives numerous end products. A complete description of these end products is, as yet, impossible; but, from work done so far upon them, it seems that different proteid bodies give rise to different compounds. Thus Pfaundler[17] obtained from serum albumin, leucine and a diamino acid, probably histidine, but from fibrin there was no leucine. He finds that the principle end products are apparently substances intermediate between peptone and amino acids. Langstein[18] has obtained from crystallized egg albumin the following substances: leucine, tryosine, glutamic acid, asparatic acid, cystine, lysine, pentamethylemdiamine, hydroxyphenylethylamine, and a polymeric carbohydrate containing N, besides a few unidentified compounds.

Numerous methods for the quantitative separation

of the first products of pepsin digestion have been worked out. They are based for the most part upon the insolubility of the compounds under different conditions. A study of the solubilities indicates the methods used for the separation.

Acid albumin is insoluble in a neutral solution, but very soluble in weak acid and alkali solutions. It precipitates from acid solution upon saturation with $MgSO_4$ and $(NH_4)_2SO_4$. Hawk and Geiss[19] have demonstrated that acid albumin can be almost completely precipitated, after digestion with an acid, upon neutralization; and as albumose and peptones are not precipitated this is a method of separation. As the albumoses are precipitated by $MgSO_4$ and $(NH_4)_2SO_4$ the latter substances can not be used to separate acid albumins from albumoses.

"Albumoses are completely precipitated by the following alkaloidal reagents:-- phosphotungstic, phosphomolybdic, picric, tannic, trichlor-acetic, and metaphosphoric acids.[20] The best method of separating them from the peptones however, is by salting them out with $(NH_4)_2SO_4$ or $MgSO_4$. Peptones do not precipitate with these salts.

Peptones are precipitated from neutral concentrated solutions by means of tannic acid and salt.[21] The albumoses having been removed, this method can be used for the estimation of peptones.

Relative Quantities of Products Produced in Acid Pepsin Digestion.

The relative quantities of albumoses, peptones, and further end products of peptic digestion produced depend entirely upon the nature of proteid digested, strength of acid pepsin, and the time and temperature of digestion. Investigators seem to agree, however, that at no time during the action is the per cent of peptones very large. Allen[22] (Commercial Organic Analysis, IV, p. 369) reports the following conclusion from the work of Chittenden. "Even with a large amount of active ferment, an abundance of free KCl, a proper temperature, and a long continued period of digestion (even five or six days), complete conversion into peptone never took place, the maximum yield being 60%. At that time it was supposed that the peptones were the final end products, and the conclusion therefore reached that the remainder of the digested material must consist of albumoses. This idea has since been proven erroneous, but the fact remains that the percentage of peptones produced is never very high.

Theory of the Chemical Action During Digestion.

Since a great many of the products of peptic digestion have been studied, a number of theories have been advanced regarding the kind of chemical change which takes place. It is

generally agreed that proteolysis is the breaking up of a complex molecule into simpler ones. The method is supposed to be by hydrolysis. Chandelon[23] subjected albumin in solution to nascent H_2O_2 and obtained substances similar to the peptones. From this he concluded that the action was hydration. As to the manner of hydration he advanced two theories: First, that the digestive action produces H_2O_2 and this in turn causes hydration; second, that the chemical structure of the pepsin molecule is similar to that of H_2O_2, namely Pu - O - O - Pu; and that this produces hydration directly. He thought the latter idea the better, but the evidence was not sufficient to be conclusive.

Effect of the Acid and the Pepsin in a Digesting Fluid, and the Most Effective Strengths of Acid Pepsin Solution.

Both the acid and the pepsin are important factors in the digestive action. Many investigators have experimented to determine just what part each played, and to show the effect of varying the quantity of each in a digestion fluid.

"The part played by the HCl in peptic digestion[24] is as yet but little understood." Goldschmidt and Lawrow maintain that HCl without pepsin will ultimately produce the same effect as if pepsin had been present; but this is probably not so.

L. Harding[25] states that a large part of the digestion action is due to HCl alone.

Allen[26] says when HCl is used alone the action goes so far as to form syntonin but not albumoses or peptones. When pepsin alone is used however, albumoses and peptones are produced as well as syntonin.

Dr. H. S. Grindley and T. Mojonnier[27] (University Studies of U. of I., April, 1903. The Artificial Method for Determining the Ease and the Rapidity of the Digestion of Meats) working upon the digestibility of raw meat, have shown that a 0.33 per cent HCl solution digested about 23.0 per cent in twenty four hours; whereas, when the acid solution contained 1.25 gram of pepsin per liter the digestion amounted to 98 per cent.

Wm. Croner[28] reports experiments which show that the most efficient digestion fluid for albumin is an acid solution in which pepsin is more than 0.1 per cent and the HCl is between .05 and 0.1 per cent.

Muller[29] concludes that only in the case of low acidity, due to HCl being combined with the proteid, is pepsin digestion improved by increased free HCl; with higher original acidity, increase of free HCl has no effect.

Brucke[30] reports experiments on fibrin in which he tested the effect of changing the strength of HCl in a digestive fluid. In a solution containing 1.15 gram HCl per liter, it took 1/2 hour to digest a flake of fibrin; and every time the acidity

was increased above this amount, the time of digestion increased. Therefore the most effective strength was 1.15 gram per liter or lower. In another experiment he showed that the most effective degree of acidity was between 0.40 and 0.80.

Mayer, Ad.[31] (Zeit. f. Biol. 17, 351-60, 1881) shows that the time required for digestion depends upon the amount of pepsin used. He says, further, that when egg albumin is digested, the most effective strength of HCl is 0.02 per cent, provided the pepsin is kept constant (1 gm-l).

Iscovesco, Henri[32] (Compt. Rend. Soc. Biol., 61, 282-84, 1906) quotes Mulder, Koopmans, and Brucke as saying that the most favorable degree of acidity in gastric juice varies according to the nature of the albumin digested. They showed also that a weak acidity favored digestion (From 0.07 per cent to 0.09 per cent solutions). His own experiments showed that with egg albumin and a 1 per cent pepsin solution an acid solution of 0.2 per cent was best. Roger and Garnier[33] (Compt. Rend. Soc. Biol., 61, 314-16) agree that an excess of HCl inhibits peptic digestion, but the optimum strength varies with the content of pepsin. They give reports from experiments showing the effect of varying both the acid and pepsin strength. A summary follows: When pepsin solution contains from ~~0~~ 0.25 to 8 grams per liter of pepsin a strength of 0.25 per cent HCl is most favorable. When a

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pepsin solution contains from 32 to 64 grams per liter, a strength of[15] 0.5 per cent HCl is most favorable. When the pepsin solution rises to 128 grams to a liter, a strength of 0.1 per cent HCl is most favorable. When the acid is weak (0.031 per cent to 0.062 per cent) the best strength pepsin solution is 8 parts in 1000.

Gautier, Armond[34] (Viry, Henri; De l'utilisation de la viande congelée l'alimentation du Soldat Lyons, 1898, p. 64) concluded, that, when the amount of pepsin is increased, the quantity of digested products also increases, although not proportionately; and that using constant absolute amounts of proteid, but decreasing the proteid concentration, the quantitative action increases, but not in proportion to the dilution of the proteid solution.

Use of Antiseptics in Artificial Digestion Experiment.

For many years it has been known that small quantities of certain substances have a marked inhibitory power on the digestive action. Among these substances are salicylic acid, benzoic acid, phenol, alcohol, alum, sodium hyposulphite, and formalin. The term antiseptics has been applied to them because they have power to prevent the growth of bacteria. For this reason some of them have been used commercially for the preservation of foods. As they not only prevent the growth of bacteria, but also destroy the power of unorganized ferments,

such as pepsin and trypsin, their action has been studied in connection with the digestion of food during its progress through the alimentary canal.

Little use of these antiseptics was made in artificial digestion experiments, however, except to determine their harmfulness as a food adulterant, until Dr. Grindley and T. Mojonnier, in their work upon the rate of digestion of meat, adopted formalin as a means of stopping the action at different periods.[35] Their purpose was to find some means of controlling the digestion, which had not gone to completion, long enough to separate the digested from the undigested portion by filtration. In this way they determined the rate of digestibility by running different samples of the same kind of meat for different periods of time. After experimenting a short time to determine the most suitable quantity of the antiseptic to use in this work, they adopted the plan of adding 10 c. c. of 40 per cent formalin to the contents of the beakers, at the time they wished to retard the action. The success of the method is shown by numerous experiments.[36]

Review of the Method of Artificial Digestion used by Grindley and Mojonnier; and brief study of its results.

It was said in the beginning that one object of the present study was to improve upon the method of artificial digestion,

formerly used in this laboratory by Dr. Grindley and T. Mojonnier (University Studies of U. of I., April, 1903. The Artificial Method for Determining the Ease and the Rapidity of the Digestion of Meats) for the purpose of determining more accurately the rates of digestion of different meats, where only small differences existed. Let us, therefore, outline that method again and briefly study its results.

1st Step. Mix sample thoroughly by passing through sausage mill three times.

2nd Step. Weigh out samples of from two to 2 1/2 grams each into Jena beakers and add 100 c. c. of an acid pepsin solution of following strength, 1.25 grams pepsin to a liter of 0.33 per cent HCl.

3rd Step. Digest for required length of time at 40° C., stirring occasionally with a glass rod.

5th Step. Add 10 c. c. of 40 per cent formalin and remove from bath.

6th Step. Filter through two carefully prepared filters one above the other. Filter paper hardened and corrugated. Wash residue with hot H_2O.

7th Step. Transfer residues and the two filter papers to Kjeldahl falsks and determine the N by the Kjeldahl method.

This having been done, the per cent undigested was calculated by dividing the amount of N found in the residue

by the amount of total N in the original sample of meat. By subtracting the per cent undigested from 100, the per cent digested was obtained.

The principle difficulties experienced in this process are these: 1. Filtration was extremely slow. In some cases it required two or three days to filter the 100 c. c. of the filtrate and wash the residues thoroughly. As a result of this slow filtration the tops of the lower filters had to be removed because it seemed that some of the soluble digestive products had crept up to the top of the paper, and resisted all efforts to wash them out. 2. The filtrates were always more or less turbid. This is accounted for by the fact that, by the method outlined, the acid albumins appear in the filtrate. Since the solution is acid after digestion, the acid albumins, or first products of proteolysis, are soluble; and the separation point between the undigested and digested portions is between the albumin and the acid albumin.

Only a few results from the many obtained by those who used this method are necessary at this point to show how reliable is the method. The following table is a portion of one made after an experiment on lean beef, round, raw, digested for one hour.37

Lab. No.	Coefficient of digestibility.
1195a	64.27
1195b	60.51
1195c	63.24
1195d	79.66
1195e	63.62
1195f	71.65
Average-----	66.69

It is evident that the figures for different portions of the same sample of meat in this table are quite variable. Whether this was due to either of the two difficulties mentioned in the preceding paragraph is not known, but the authors of the method thought that with more skill in manipulation, more uniform results could be obtained. Later work showed this to be the case, but the desired uniformity was seldom obtained in the digestion of raw meat.

EXPERIMENTAL WORK.

Experiment 1.

The first experimental work done in the present study was to make a comparison of the rate of digestibility of poultry kept in cold storage for several months in an undrawn condition; and that which had been stored after being drawn. The object was two fold, first to make the comparison just mentioned;and second,to test the effectiveness of a slight change in the kind of filters used.

Preparation of Samples. The meat was cut as completely as possible from six undrawn chickens which were in a frozen condition. When all the bone and gristle had been separated from the flesh, the latter was thoroughly mixed by passing it three times through a sausage mill. A small representative portion was then packed in a mason jar and labelled 2111. Half a dozen drawn chickens were then sampled in the same way and labelled 2112.

Method. The method used in this experiment was the same as that outlined on page 17except for a modification in the kind of filters and in the strength of acid pepsin solution. Instead

of using hardened quantitative paper in both the upper and lower funnels it was placed only in the lower one. The upper funnel was fitted with an ordinary quantitative filter paper and in this was placed a little cotton. Cotton also was pressed into the short stem of this funnel. It was thought such an arrangement might hasten the filtration and produce a filtrate as clear as by the oldmethod. The acid pepsin solution contained 1 gram of pepsin (MERCK)to a liter of N/10 HCl.

Sixteen samples of meat from each jar were weighed out and digested. The first part of the experiment (a) was made upon the undrawn, and the second (b) upon the drawn chicken. In each case four samples were digested for 1 hour (Nos. 2111/1 to 2111/4 and 2112/1 to 2112/4 inclusive); four for 2 hours (Nos. 2111/5 to 2111/8 and 2112/5 to 2112/8 inclusive); four for six hours (Nos. 2111/9 to 2111/12 and 2112/9 to 2112/12 inclusive); and four for 12 hours (Nos. 2111/12 to 2111/16 and 2112/13 to 2112/16 inclusive). The results are given in table 1.

Observations and conclusions. From the results there given it seems that the meat from the undrawn poultry digested a little more rapidly than that from the drawn poultry. At the end of the first hour the difference was about 2 per cent, at the end of the second hour, 4 per cent, at the end of the

Table 1.

Rate of digestion of drawn and undrawn poultry.

Experiment 1.

Exp. No.	Lab. No.	Kind of meat.	Time of digestion.	Per cent of digested N.
1a	2111/1	Chicken, undrawn, frozen	1 hr.	84.20
"	2111/2	" " "	1 "	-----
"	2111/3	" " "	1 "	89.49#
"	2111/4	" " "	1 "	83.87
	Average			84.04
"	2111/5	" " "	2 "	-----
"	2111/6	" " "	2 "	72.37
"	2111/7	" " "	2 "	88.29
"	2111/8	" " "	2 "	87.71
	Average			82.79
"	2111/9	" " "	6 "	98.77
"	2111/10	" " "	6 "	-----
"	2111/11	" " "	6 "	91.21
"	2111/12	" " "	6 "	-----
	Average			94.99
"	2111/13	" " "	12 "	94.01
"	2111/14	" " "	12 "	-----
"	2111/15	" " "	12 "	-----
"	2111/16	" " "	12 "	93.28
	Average			93.65
1b	2112/1	" drawn "	1 "	81.59
"	2112/2	" " "	1 "	-----
"	2112/3	" " "	1 "	83.08
"	2113/4	" " "	1 "	81.26
	Average			81.97

This number is not included in the numbers averaged.

Table 1.

Number of drones and workers per hive.

No.	No.	Kind of Comb.	Number of Drones.	Number of Workers.
1	1	Worker	1	1
2	2	Worker	2	2
3	3	Worker	3	3
Average			2	2
4	4	Drone	4	4
5	5	Drone	5	5
6	6	Drone	6	6
Average			5	5
7	7	Worker	7	7
8	8	Worker	8	8
9	9	Worker	9	9
Average			8	8
10	10	Drone	10	10
11	11	Drone	11	11
12	12	Drone	12	12
Average			11	11

Table 1.

Rate of digestion of drawn and undrawn poultry.

Experiment 1.

Exp. No.	Lab. No.	Kind of meat.	Time of digestion.	Per cent of digested N.
1b	2112/5	Chicken, drawn, frozen	2 hrs.	79.23
"	2112/6	" " "	2 "	94.33#
"	2112/7	" " "	2 "	80.54
"	2112/8	" " "	2 "	76.09
Average				78.62
"	2112/9	" " "	6 "	92.54
"	2112/10	" " "	6 "	-----
"	2112/11	" " "	6 "	92.90
"	2112/12	" " "	6 "	-----
Average				92.67
"	2112/13	" " "	12 "	-----
"	2112/14	" " "	12 "	93.22
"	2112/15	" " "	12 "	94.62
"	2112/16	" " "	12 "	89.74
Average				92.53

This number is not included in the numbers averaged.

Table 1.

Date of flowering and number bolting.

sixth hour, 2 per cent, and at the end of the 12th hour, 1 per cent. There was no digestive action between the sixth and twelfth hours. Presumably therefore the digestion was completed at the end of the former period.

It is not intended however to draw conclusions of this kind from this experiment. It is evident that the variations in the duplicates are in some cases as much as the differences between the drawn and undrawn poultry. Furthermore a sufficient number of results were not obtained to give any reliable information as to the relative rates of digestion. The experiment was disappointing from a mechanical standpoint because the change in the filters did not prove successful in hastening filtration. It was entirely too slow to be practicable, and the filtrates were all turbid. Another difficulty was experienced in Kjeldahling the residues. The lerge amount of cellulose due to the cotton and two filter papers caused trouble.

Experiment 2.

After the first experiment attempts were made to contrive some arrangement by which the difficulties in filtration could be overcome. None was successful however and in the second experiment the same method was used as in experiment 1, except that the cotton was discarded.

Object. The object of experiment 2 was to determine two things: first the effect of varying the strength of the digestive fluid; and second the relative value of formalin and phenol as an antiseptic. The purpose of changing the strength of acid was to retard the action and thus show wider differences in the amount digested after each interval of time. The use of a new antiseptic was not necessary, since formalin had been found satisfactory but, as phenol had been suggested it was tried.

Method. Thirty two samples of beef (round, lean) were used in this experiment. They were all taken from the sample labelled 2120. The experiment was divided into four parts a, b, c, and d. Section a included laboratory numbers 2120/1 to 2120/8, section b numbers 2120/17 to 2120/24, section c numbers 2120/41 to 2120/48, and section d numbers 2120/49 to 2120/56. Section a was conducted as follows. Four samples were weighed out in the usual manner and treated with 100 c. c. of acid pepsin solution containing 1 gram of pepsin to 1 liter of N/10 HCl. They were digested for one hour, treated with formalin and filtered as quickly as possible. Four more samples were treated with the same strength acid pepsin solution, digested for 2 hours, treated with formalin and filtered as before. In section b also, eight samples were used.

Four of them were treated with 100 c. c. of an acid pepsin solution containing 1/2 gram of pepsin to a liter of N/50 HCl. They were digested for one hour, treated with formalin and filtered immediately. The other four were treated in the same manner but were digested two hours instead of one. Section c was conducted the same as section a except that phenol was used in place of formalin. Section d was carried on in a little different way. Four of the samples were treated as the first four of c but instead of the contents of the beakers being filtered immediately after the phenol was added, they were allowed to stand for 24 hours before filtering. The other four samples were treated as the second four in a but here also the filtration was not begun until 70 hours after formalin had been added. The results of this operation are shown in table 2.

Observations and Conclusions. It is evident from the results of a and b that there was a decrease in the digestibility at the end of the first hour, when the acid pepsin solution was weakened, of about 10%. At the end of the second hour an increase in digestibility appeared but as only one out of four results was obtained it can not be relied upon. Although the results here given are too few to warrant us in drawing any general conclusions concerning the effect of different strengths of the digestive fluid, they indicate that the weaker of the two solutions used

Table 2.

Change of strength of acid pepsin solution and comparison of formalin and phenol as antiseptics.

Experiment 2.

Exp. No.	Lab. No.	Strength of Acid.	Strength of Pepsin.	Time of digestion.	Antiseptic used.	Time of standing before filtering.	Per cent of digested nitrogen.
2a	2124/1	N/10	1 gm-l	1 hr.	Formalin	None	75.68
"	2124/2	"	"	1 "	"	"	79.69
"	2124/3	"	"	1 "	"	"	72.65
"	2124/4	"	"	1 "	"	"	78.74
	Average						76.69
"	2120/5	"	"	2 "	"	"	81.63
"	2120/6	"	"	2 "	"	"	80.12
"	2120/7	"	"	2 "	"	"	80.33
"	2120/8	"	"	2 "	"	"	81.66
	Average						80.93
2b	2120/17	N/50	1/2 gm-l	1 hr.	"	"	67.17
"	2120/18	"	"	1 "	"	"	-----
"	2120/19	"	"	1 "	"	"	60.82
"	2128/20	"	"	1 "	"	"	67.04
	Average						65.01
"	2120/21	"	"	2 hrs.	"	"	-----
"	2120/22	"	"	2 "	"	"	-----
"	2120/23	"	"	2 "	"	"	85.55
"	2120/4	"	"	2 "	"	"	-----
	Average						85.55
2c	2120/41	N/10	1 gm-l	1 hr.	Phenol	"	-----
"	2120/42	"	"	1 "	"	"	73.87
"	2120/43	"	"	1 "	"	"	-----
"	2120/44	"	"	1 "	"	"	-----
	Average						73.87

Table 5.

Effect of strength of acid solution and temperature of formation and removal of hydrosulfide.

Experiment 5.

Exp. No.	Conc. of Acid.	Strength of Solution.	Time of Exposure.	Additional Time of Heating.	Temperature of Oil-Bath.	Time of Exposure at 110°.	Percentage of Sulfur.
25	1/10	1 gm-l	1 hr.	3 hrs.	110°	3 hrs.	0.00
26	1/10	1 gm-l	1	3	110	3	0.00
27	1/10	1 gm-l	1	3	110	3	0.00
28	1/10	1 gm-l	1	3	110	3	0.00
29	1/10	1 gm-l	2	3	110	3	0.00
30	1/10	1 gm-l	2	3	110	3	0.00
31	1/10	1 gm-l	2	3	110	3	0.00
32	1/10	1 gm-l	2	3	110	3	0.00
33	1/10	1 gm-l	3	3	110	3	0.00
34	1/10	1 gm-l	3	3	110	3	0.00
35	1/10	1 gm-l	3	3	110	3	0.00
36	1/10	1 gm-l	3	3	110	3	0.00
37	1/10	1 gm-l	4	3	110	3	0.00
38	1/10	1 gm-l	4	3	110	3	0.00
39	1/10	1 gm-l	4	3	110	3	0.00
40	1/10	1 gm-l	4	3	110	3	0.00

Table 2.

Change of strength of acid pepsin solution and comparison of formalin and phenol as antiseptics.

Experiment 2.

Exp. No.	Lab. No.	Strength of Acid.	Strength of Pepsin.	Time of digestion.	Antiseptic used.	Time of standing before filtering.	Per cent of digested nitrogen.
2c	2120/45	N/10	1 gm-l	2 hrs.	Phenol	None	81.52
"	2120/46	"	"	2 "	"	"	99.46#
"	2120/47	"	"	2 "	"	"	78.35
"	2120/48	"	"	2 "	"	"	-----
	Average						79.93
2d	2120/49	"	"	1 hr.	"	24 hrs.	73.80
"	2120/50	"	"	1 "	"	24 "	73.40
"	2120/51	"	"	1 "	"	24 "	76.59
"	2120/52	"	"	1 "	"	24 "	75.99
	Average						74.94
"	2120/53	"	"	2 hrs.	Formalin	70 "	95.56
"	2120/54	"	"	" "	"	70 "	-----
"	2120/55	"	"	" "	"	70 "	95.62
"	2120/56	"	"	" "	"	70 "	95.09
	Average						95.42

This number is not included in the numbers averaged.

Table .

Showing of [illegible] of acid-resin solution and [illegible] of
[illegible] and physical properties.

Specimens E.

Exp. No.	Lbs. No.	Acid.	Time of dissolving.	Lbs.	Time of [illegible]

has a tendency to retard the action for the first hour.

As to the comparative value of formalin and phenol as an antiseptic, sections a and c show there is little difference. In one hour with formalin an average of 76.69 per cent was digested and with phenol 73.87. In two hours with formalin 80.93 was digested and with phenol 79.93. Furthermore in d it is shown that when phenol was used and the solutions allowed to stand 24 hours before filtering the amount digested averaged 74.94. This indicates that the phenol had been effective in completely stopping the action at the end of the second hour. When formalin was used and the solutions allowed to stand 70 hours before filtration the results show that digestion must have continued after the formalin was added. It has formerly been shown however, that formalin is perfectly satisfactory in preventing the action for twenty four hours. During the progress of the experiment it was noticed that a light precipitate seemed to form in the solution upon the addition of phenol. The latter substance, moreover, seemed a little more troublesome during the mechanical manipulations than formalin and for these reasons it was not used in later experiments.

The results of experiment 2, like those of experiment 1, are somewhat disappointing because the duplicates in many cases do not check. Until this feature is corrected it is impossible.

to draw conclusions from the figures. In this experiment also the same difficulties in filtering as before were experienced.

Experiment 3.

Since the method used in the previous experiments had proven impracticable because of the difficulty in filtering, and the inaccuracies in results, some change was desired to eliminate these difficulties.

No progress was made toward a remedy however, until it was suggested, by Dr. Grindley, that the solution be neutralized before filtration. This, he thought might hasten filtration, and also would represent more nearly the conditions actually existing in natural digestion. Furthermore a study of the methods of artificial digestion of the earlier investigators shows that in nearly all cases they separated the undigested portions after neutralization. Such a process is a radical departure from the method used previously in the first two experiments of this study. It will be remembered that during the progress of digestion some of the albumin is converted into syntonium (acid albumin), albumose, and peptone, as well as to further end products. When the solution is filtered without neutralization, as has been described the insoluble albumin

is separated from the acid albumins, albumoses, and peptones. The albumoses and peptones being very soluble filter rapidly but the acid albumins being of much greater molecular weight cause the pores of the filter to become clogged and inhibit filtration. Furthermore they cause turbidity of the filtrate. But if the solutions were neutralized before filtration different conditions would exist. The acid of the acid albumin being neutralized, the proteid would precipitate and become insoluble in the neutral solution. The albumoses and peptones would filter through as before and the separation point instead of being between the albumin and acid albumin would be between the albumin and albumose. Theoretically the neutralization should materially hasten filtration and eliminate the turbidity of the filtrate. The objection might be raised at this point of course that neutralization would destroy the value of the experiments since it would not parallel the conditions existing in the stomach. There is no reason however for saying that without neutralization the conditions in the stomach are more nearly obtained than with neutralization. In no case can the conditions in artificial experiments be made exactly parallel to those of the alimentary tract, so the best method which approximates these conditions is most valuable. The term digestion is vague and indefinite, and there is no reason

why the neutralization method should not be called digestion as well as the other. In this connection Allen says:[38] 'There has commonly been no marked distinction drawn between the mere conversion of albumin into syntonin or other soluble forms and the true peptonization characteristic of the action of pepsin, both these changes having been confounded under the term digestion.' In this experiment therefore the solutions were neutralized after digestion as described below.

Object. The object of experiment 3 was to compare the results of the neutralization method with those formerly obtained, and the effect of HCl alone and the acid pepsin solution.

Method. Ten samples of lean beef (round) were weighed out as usual and labelled from 2120/81 to 2120/90 inclusive. The first five were treated with 100 c. c. of N/10 HCl but no pepsin. Number 2120/81 was digested for 1 hour, number 2120/12 for 2 hours, 2120/83 for 6 hours, 2120/84 for 12 hours, and 2120/85 for 24 hours. At the end of digestion 10 c. c. of formalin were added to each and the solutions neutralized with KOH to litmus paper. Normal KOH was added until the solution was nearly neutralized and then the operation was completed with a N/50 KOH solution. After being neutralized the contents of the beakers were filtered. The remaining five samples were treated with 100 c. c. of an acid pepsin solution containing 1 gram of pepsin per liter of N/10 HCl. They were

Table 3.

Comparison of acid alone and acid pepsin solution as digestive fluid.

Experiment 3.

Exp. No.	Lab. No.	Strength of Acid.	Strength of Pepsin.	Antiseptic used.	Time of digestion.	Per cent of nitrogen digested.
3	2120/81	N/10	None	Formalin	1 hr.	14.15
"	2120/82	"	"	"	2 "	-----
"	2120/83	"	"	"	6 "	20.49
"	2120/84	"	"	"	12 "	14.12
"	2120/85	"	"	"	24 "	13.02
"	2120/86	"	1 gm-l	"	1 "	79.38
"	2120/87	"	"	"	2 "	87.06
"	2120/88	"	"	"	6 "	92.10
"	2120/89	"	"	"	12 "	94.32
"	2120/90	"	"	"	24 "	95.58

Experiment 7.

Exp. No.	Lab. No.	Weight of Acid.	Acidity to Penolph.	Time of Exposure.	Per cent of Nitrogen Liberated.

digested as follows:-- No. 2120/86 for 1 hour, 2120/87 for 2 hours, 2120/88 for 6 hours, 2120/89 for 12 hours, and 2120/90 for 24 hours. These also were neutralized after the addition of formalin and then filtered. It was found upon filtering, that the double filter was not necessary in order to make the filtrate almost clear, so only one funnel, containing a quantitative filter paper, was used. With the exception of the modifications here mentioned the method was the same as in the previous experiment.

Observations and Conclusions. From the table it is evident that the HCl alone digested only a small amount in one hour and that this except in one case, was not increased by longer digestion. As no duplicates were run in this experiment, the results cannot be depended on, but the similarity of the digested portion for the 1, 12, and 24 hour periods indicates that the digestion does not increase. An explanation of this can easily be made. HCl acts upon albumin and produces acid albumin, but the action goes no further. Then the solution is neutralized and the albumin is again precipitated.

The results with the acid pepsin solution show that digestion progresses from approximately 80% in one hour to 95% in 24 hours. The results are similar to those obtained in experiments 1 and 2a. They show therefore two things: first, that the new method in which neutralization is accomplished gives

a coefficient of digestibility as high as without neutralization and, second that the pepsin added to the acid solution increases the digestibility from about 14% in one hour to approximately 80%. If the first of these conclusions is true it is also evident that with acid pepsin solution, the quantity of acid albumin present at any time is small. Otherwise, upon neutralization, there would be a material decrease in the amount digested and an increase in the amount undigested.

Considerable encouragement was drawn from this experiment on account of the rapidity of filtration. Since the acid albumin was precipitated by neutralization, the solutions filtered quite rapidly. At the same time the filtrates were not very turbid and it was thought with more perfect neutralization they could be made entirely clear. On the whole, therefore, it was considered a great improvement to neutralize the solutions after digestion.

Experiment 4.

Since a method had been obtained which seemed to be fairly satisfactory as regards accuracy and quite acceptable as regards speed, an extensive experiment was planned.

Object. The object of experiment 4 was to determine the differences in the rate of digestibility of the wholesale

cuts of beef.

Method. A prime steer, 2 years old had been slaughtered for analytical purposes and one half of the animal divided into wholesale cuts. A representative sample from each of 9 of the cuts was taken for digestion work. The cuts and their laboratory numbers were as follows: Round No. 2134, loin No. 2136, rib No. 2137, flank No. 2138, plate No. 2139, Chuck No. 2140, clod No. 2141, neck No. 2142, fore shank No. 2143. Samples of from two to 2.5 grams of meat from each cut were taken and treated with an acid pepsin solution containing 1 gram of pepsin per liter of 0.2 per cent HCl. It was planned to digest three samples from each cut for 1 hour, three for two hours, three for four hours, and three for 12 hours. In some cases, however, the 2 hour, 4 hour, and 12 hour periods were ommitted as the table shows. In every case the samples digested gor 1 hour were numbered from 1 to 3 inclusive, the two hour samples from 4 to 6 inclusive, the 4 hour samples from 7 to 9 inclusive, and the 12 hour samples from 10 to 12 inclusive. For instance the samples of the loin digested for one hour included 2136/1 and 2136/3, for two hours 2136/4 and 2136/6, for 4 hours 2136/7 and 2136/9, and for 12 hours 2136/10 and 2136/12. The manipulation was the same as in experiment 3, except that neutralization of the digested solution was more carefully done. This

was accomplished by heating the beakers on the water bath at 90° and neutralizing them while hot. The KOH solution was added from a pipette drop by drop until the solution was neutral to litmus paper. In most cases, after neutralization the precipitated and insoluble proteids coagulated leaving a clear liquid instead of a turbid one as had been obtained without heating on the water bath. Another slight change in the method was the elimination of the formalin. It was not used because it was thought the neutralization would prevent all action of the HCl and heating to 90° would destroy the power of the pepsin. After

the contents of the beakers had been heated for about 1/2 hour, they were filtered through a single quantitative filter paper while hot. Filtration was rapid. In a great many cases the the filtrates were not quite clear and some of these were refiltered. The remainder of the processes was carried on were the same as before, and the results of the experiment are shown in table 4.

Observations and Conclusions. A study of the table shows some very peculiar results. The round for example was much more easily digestible than the loin because it took four hours to digest as much of the latter as was digested in one hour by the former. The rib digested at about the same rate as the round and the flank was midway between that and the loin.

Table 4.

Ratio of digestion of different wholesale cuts of beef.

Experiment 4.

Exp. No.	Lab. No.	Wholesale cut.	Strength of Acid. Per cent.	Strength of Pepsin.	Time of digestion.	Per cent of digested nitrogen.
4	2134/1	Round	0.2	1 gm-l	1 hr.	87.75
"	2134/2	"	"	"	1 "	88.93
"	2134/3	"	"	"	1 "	89.63
Average						88.77
"	2134/4	"	"	"	2 hrs.	91.20
"	2134/5	"	"	"	2 "	90.66
"	2134/6	"	"	"	2 "	92.85
Average						91.57
"	2134/7	"	"	"	4 "	91.94
"	2134/8	"	"	"	4 "	92.96
"	2134/9	"	"	"	4 "	93.76
Average						92.88
"	2136/1	Loin	"	"	1 hr.	62.81
"	2136/2	"	"	"	1 "	68.88
"	2136/3	"	"	"	1 "	-----
Average						65.84
"	2136/4	"	"	"	2 hrs.	64.97
"	2136/5	"	"	"	2 "	65.28
"	2136/6	"	"	"	2 "	67.29
Average						65.84
"	2136/7	"	"	"	4 "	-----
"	2136/8	"	"	"	4 "	89.91
"	2136/9	"	"	"	4 "	-----
Average						89.91

Table 4.

Data of Legislation of different municipalities into of Court.

Experiment 4.

Exp. No.	Leg. In.	Municipalities	Strength of Acid. Phen.	Time of Ab-	Per cent
		Com.			
1	[illegible]	Lorum	[illegible]	1 hr.	87.12
2	[illegible]	[illegible]	[illegible]	1	88.93
3	[illegible]	[illegible]	[illegible]	1	[illegible]
Average					
4	[illegible]	[illegible]	[illegible]	2 hrs.	91.80
5	[illegible]	[illegible]	[illegible]	2	[illegible]
6	[illegible]	[illegible]	[illegible]	2	[illegible]
Average					
7	[illegible]	[illegible]	[illegible]	4	[illegible]
8	[illegible]	[illegible]	[illegible]	4	[illegible]
9	[illegible]	[illegible]	[illegible]	4	93.72
Average					
10	[illegible]	Lorum	[illegible]	1 hr.	[illegible]
11	[illegible]	[illegible]	[illegible]	1	[illegible]
12	[illegible]	[illegible]	[illegible]	1	[illegible]
Average					
13	[illegible]	[illegible]	[illegible]	2 hrs.	[illegible]
14	[illegible]	[illegible]	[illegible]	2	[illegible]
15	[illegible]	[illegible]	[illegible]	2	[illegible]
Average					
16	[illegible]	[illegible]	[illegible]	4	[illegible]
17	[illegible]	[illegible]	[illegible]	4	[illegible]
18	[illegible]	[illegible]	[illegible]	4	[illegible]
Average					

Table 4.

Ratio of digestion of different wholesale cuts of beef.

Experiment 4.

Exp. No.	Lab. No.	Wholesale cut.	Strength of Acid. Per cent.	Strength of Pepsin.	Time of digestion.	Per cent of digested nitrogen.
4	2136/10	Loin	0.2	1 gm-l	12 hrs.	88.97
"	2136/11	"	"	"	12 "	91.13
"	2136/12	"	"	"	12 "	77.53#
Average						90.05
"	2137/1	Rib	"	"	1 hr.	87.85
"	2137/2	"	"	"	1 "	88.09
"	2137/3	"	"	"	1 "	-----
Average						87.97
"	2137/4	"	"	"	2 "	89.58
"	2137/5	"	"	"	2 "	88.13
"	2137/6	"	"	"	2 "	87.75
Average						88.48
"	2137/7	"	"	"	4 "	90.02
"	2137/8	"	"	"	4 "	89.40
"	2137/9	"	"	"	4 "	90.37
Average						89.93
"	2138/1	Flank	"	"	1 "	79.67
"	2138/2	"	"	"	1 "	66.96
"	2138/3	"	"	"	1 "	82.40
Average						81.03
"	2138/4	"	"	"	2 "	85.56
"	2138/5	"	"	"	2 "	83.19
"	2138/6	"	"	"	2 "	82.95
Average						83.90
"	2138/7	"	"	"	4 "	-----
"	2138/8	"	"	"	4 "	90.70
"	2138/9	"	"	"	4 "	90.88
Average						90.79

This number is not included in the numbers averaged.

Table 4.

Ratio of digestion of different wholesale cuts of beef.

Experiment 4.

Exp. No.	Lab. No.	Wholesale cut.	Strength of Acid. Per cent.	Pepsin.	Time of di-gestion.	Per cent of digested nitrogen.
4	2139/1	Plate	0.2	1 gm-1	1 hr.	85.27
"	2139/2	"	"	"	1 "	82.90
"	2139/3	"	"	"	1 "	-----
Average						84.09
"	2139/4	Plate	"	"	2 "	91.11
"	2139/5	"	"	"	2 "	87.82
"	2139/6	"	"	"	2 "	87.29
Average						88.74
"	2139/7	"	"	"	4 "	87.04
"	2139/8	"	"	"	4 "	89.88
"	2139/9	"	"	"	4 "	89.85
Average						88.92
"	2140/1	Chuck	"	"	1 "	-----
"	2140/2	"	"	"	1 "	86.18
"	2140/3	"	"	"	1 "	87.10
Average						86.64
"	2140/4	"	"	"	2 "	90.07
"	2144/5	"	"	"	2 "	88.84
"	2140/6	"	"	"	2 "	90.87
Average						89.92
"	2141/1	Clod	"	"	1 "	12.15
"	2141/2	"	"	"	1 "	------
"	2141/3	"	"	"	1 "	21.85
Average						17.00
"	2142/1	Neck	"	"	1 "	82.88
"	2142/2	"	"	"	1 "	80.33
"	2142/3	"	"	"	1 "	84.10
Average						82.43

Table 4.

Ratio of digestion of different wholesale cuts of beef.

Experiment 4.

Exp. No.	Lab. No.	Wholesale cuts.	Strength of Acid. Per cent.	Pepsin.	Time of digestion.	Per cent of digested nitrogen.
4	2142/4	Neck	0.2	1 gm-1	2 hrs.	90.30
"	2142/5	"	"	"	2 "	89.40
"	2142/6	"	"	"	2 "	87.70
Average						89.13
"	2143/1	Fore shank	"	"	1 "	77.68
"	2143/2	"	"	"	1 "	82.91
"	2143/3	"	"	"	1 "	-----
Average						80.29
"	2143/4	"	"	"	2 "	85.76
"	2143/5	"	"	"	2 "	83.69
"	2143/6	"	"	"	2 "	82.25
Average						83.90
"	2143/7	"	"	"	4 "	91.59
"	2143/8	"	"	"	4 "	92.55
"	2143/9	"	"	"	4 "	90.31
Average						91.48

The plate compared favorably with the round as did the chuck, while the neck and fore-shank acted similarly to the flank. In the case of the clod the per cent digested was extremely low and variable. It is not intended however to draw such conclusions from the results of this experiment. The figures obtained here will be interesting to compare with those obtained in future experiments of this kind, but aside from that they are of little value.

It was thought at first that an explanation of some of the unexpected results might be in the fact that some of the wholesale cuts contained a large amount of fat and this retarded digestion and caused the triplicates to vary. It was noticed however that the cuts which were lean digested no more readily than those which were fat, and the triplicates were no more uniform.

It had been expected that precipitation of the acid albumin by neutralization would eliminate the cause of variation in the duplicates, for two reasons. In the first place, a cleaner separation could be made between the digested and undigested portions than before, and in the second place the rapidity of filtration would tend to decrease the error in manipulation. Although the triplicates given in the table for this experiment are fairly close, however, there is more variation than was desired. The reason for such variation

therefore was laid to imperfect neutralization. That is, it was supposed, the same neutralization point had not been produced in the different beakers and consequently the same per cent of acid albumin had not been precipitated from the various solutions. This hypothesis was strengthened by the fact that there was some turbidity in filtrates which, even upon refiltering, could not be removed. The acid albumin which produced this turbidity, it was argued, was variable in quantity in the different filtrates and consequently caused variation in the results.

Experiment 5.

Object. Since in previous experiments the coefficient of digestion for one hour had been in nearly all cases above 75 per cent the purpose of this experiment was to reduce the time of the first period.

Method. Twelve samples of beef (lean, round) were weighed out and treated with 100 c. c. of an acid pepsin solution containing 1 gram of pepsin per liter of 0.2 HCl. The laboratory number of the beef was 2170. Three samples (Nos. 2170/1 to 2170/3 inclusive) were digested for 1/2 hour, six (Nos. 2170/4 to 2170/6 and 2170/10 to 2170/12 inclusive) were digested for 1 hour, and three (Nos. 2170/7 to 2170/9 inclusive) were digested for 1 1/2 hours. The manipulation was the same as in the previous experiment and great care was exercised during the neutralization. The results are given in table 5.

Observations and Conclusions. Contrary to expectations the per cent digested in 1/2 hour is very high, higher than some previous figures for one hour digestion. It is evident, therefore, that the digestive action with an acid pepsin solution of the strength used here, is extremely rapid. This is further emphasized by the results in the first set which were digested

Table 5.

Rate of digestion of a sample of beef.

Experiment 5.

Exp. No.	Lab. No.	Strength of Acid.	Strength of Pepsin.	Time of digestion.	Per cent of digested nitrogen.
5	2170/1	0.2	1 gm-l	1/2 hr.	89.26
"	2170/2	"	"	"	88.42
"	2170/3	"	"	"	88.15
Average					88.61
"	2170/4	"	"	1 hr.	92.72
"	2170/5	"	"	"	89.41
"	2170/6	"	"	"	-----
Average					91.07
"	2170/10	"	"	"	87.93
"	2170/11	"	"	"	88.00
"	2170/12	"	"	"	84.37
Average					86.73
"	2170/7	"	"	1 1/2 hr.	-----
"	2170/8	"	"	"	87.40
"	2170/9	"	"	"	94.04
Average					90.72

The digestion seems to be as complete at the end of one hour as at the end of 1 1/2 hours. The results for the second set digested for one hour,

for one hour, however, are considerably reduced. An explanation for this discrepancy can not be given.

It is evident again, in spite of the utmost care at neutralization, that the variation in the triplicates was larger than it should have been.

Experiment VI.

Numerous attempts were made after the last experiment to find an indicator which would be more delicate than litmus paper when the solutions were neutralized after digestion. None was found however, so in this experiment a different neutralizing agent was tried.

Object. The purpose of the experiment was to compare $BaCO_3$ with KOH as a means of neutralization.

Method. The beef used in this case was labelled 2184. Two samples were digested for 15 minutes, two for 1/2 hour, two for 1 hour, two for 2 hours and two for 6 hours. One of the two in each case was neutralized with KOH as before and the other with $BaCO_3$ (Kahlbaum). The method of using the latter was to weigh out about 1 1/2 grams each in small watch glasses and pour the different portions into the proper beakers at the right time. The strength of acid pepsin solution and method

Table 6.

Rate of digestion of a sample of beef. Comparison of KOH and $BaCO_3$ as neutralizing agent.

Experiment 6.

Exp. No.	Lab. No.	Strength of Acid.	Strength of Pepsin.	Time of digestion.	Neutralizing agent.	Per cent of digested N.
6	2184/1	0.2	1 gm-l	1/4 hr.	KOH	79.50
"	2184/2	"	"	"	$BaCO_3$	80.43
"	2184/3	"	"	1/2 hr.	KOH	90.32
"	2184/4	"	"	"	$BaCO_3$	86.75
"	2184/5	"	"	1 hr.	KOH	88.25
"	2184/6	"	"	"	$BaCO_3$	87.76
"	2184/7	"	"	2 hrs.	KOH	91.24
"	2184/8	"	"	"	$BaCO_3$	83.64
"	2184/9	"	"	6 hrs.	KOH	97.52
"	2184/10	"	"	"	$BaCO_3$	94.80

of manipulation were the same as in the previous experiment.

The results are shown in table 6.

Observations and Conclusions. Here again the digestion was remarkably high at the end of 15 minutes and can not be satisfactorily explained in the light of former results on the digestibility of raw beef. The duplicates in this series were close enough in most cases to show that $BaCO_3$ would make as good a neutralizing agent as KOH. Its advantages were at least two in number. In the first place it produced the same degree of alkalinity in each sample. This was the condition most desired because if the same conditions were present in all the samples as regards neutrality, the duplicates should check even though the very best neutralization point for the precipitation of all the acid albumin was not reached. In the second place the tedious work of adding just the proper amount of KOH was eliminated. It was decided therefore to use $BaCO_3$ in later experiments.

Experiment 7.

Object. The object of experiment 7 was to reduce the initial period of digestion still further and to make further tests of the value of $BaCO_3$ as a neutralizing agent.

Method. The beef used was labelled 2185. Eight samples were weighed out and digested in duplicate for the following

Table 7.

Rate of digestion of sample of beef.

Experiment 7.

Exp. No.	Lab. No.	Strength of Acid. Per cent.	Pepsin.	Time of digestion.	Neutralizing agent.	Per cent of digested N.
7	2185/1	0.2	1 gm-1	10 min.	$BaCO_3$	64.70
"	2185/2	"	"	"	"	62.10
Average						63.40
"	2185/3	"	"	20 min.	"	74.07
"	2185/4	"	"	"	"	73.63
Average						73.85
"	2185/5	"	"	30 min.	"	80.11
"	2185/6	"	"	"	"	-----
Average						80.11
"	2185/7	"	"	60 min.	"	86.14
"	2185/8	"	"	"	"	87.33
Average						86.73

periods of time: 10 minutes, 20 minutes, 30 minutes, and 60 minutes. The conditions and method of manipulation were the same as in the previous experiment except that all the solutions were neutralized with $BaCO_3$ after digestion.

The results are tabulated on the next page.

Observations and Conclusions. While the results given here are not sufficient in number to prove the efficiency of $BaCO_3$, they are quite uniform and indicate an improvement over the KOH. The surprising fact again is the large coefficient of digestion during the first period of 10 minutes. This makes it still more evident that an acid pepsin solution containing 1 gram of pepsin to a liter of 0.2 per cent HCl digests raw meat with great rapidity.

Conclusions from Results Obtained thus far, in regard to the method of conducting the experiments.

1. The old method of filtering the solutions after digestion without neutralization is tedious, cumbersome, and inaccurate.

2. Neutralization after digestion eliminates the difficulties connected with filtration.

3. Both formalin and phenol are effective antiseptics

but neither is necessary when the neutralization method is used.

4. Neutralizing the solutions before filtering does not seem to increase the uniformity of results to any great extent.

5. $BaCO_3$ is as effective a neutralizing agent as KOH and much more convenient.

6. An acid pepsin solution containing 1 gram of pepsin per liter of N/10 HCl or of 0.2 per cent HCl digests raw beef with great rapidity. When the strength of the digestive fluid is reduced to 1/2 gram of pepsin per liter of N/50 HCl the rapidity of the action is reduced but is still high.

7. When N/10 HCl alone is used as the digestive agent, the per cent digested is very low at the end of the first hour and does not increase with longer digestion.

Experiment 8.

Bearing in mind what was learned in the previous work, experiment 8 was planned on a more extensive scale than any previous series.

Object. The object of the experiment was to compare the digestibility of a sample of beef in different digestive fluids, the time of digestion being kept constant. The experiment was divided into 8 parts, a, b, c, d, e, f, g, and h. The purposes of each part was as follows:

(a) To digest five samples with an acid pepsin solution containing 1/2 gram of pepsin per liter of N/20 HCl and determine the per cent digested, the per cent undigested and the per cent of albumoses produced.

(b) To repeat (a) using the N/20 acid alone instead of the acid pepsin.

(c) To repeat (a) with water alone as the digesting agent.

(d) To repeat (a) using a pepsin solution of 1/2 gram of pepsin to a liter of H_2O. No acid.

(e) To repeat (a) using an acid pepsin solution containing 1/2 gram of pepsin to a liter of N/40 HCl.

(f) To repeat (a) using an acid pepsin solution containing 1/2 gram of pepsin to 1 liter of N/100 HCl.

(g) To repeat (a) using no meat.

(h) To make a water extract of the meat and with the residues repeat (a), (b), and (c).

Method. The method in general for this experiment was the same as that used for experiment 7. $BaCO_3$ was used to neutralize the solution after digestion. Instead of using the dry salt, however, as in the previous work, a $BaCO_3$ paste was prepared for this work by Mr. H. H. Mitchell. In all cases 2 c. c. of the paste was used to neutralize the solution. The time of digestion of all samples was one hour. As it was desired to obtain results for the per cent digested, the per cent undigested and the per cent of albumoses in each section of this experiment the first were always lettered x, the second z, and the third y. Each section however was given a different lab. no.

Method for (a). Five samples of beef were weighed out as usual, and labelled from 2186/1 to 2186/5 inclusive. They were digested for one hour in an acid pepsin solution containing 1/2 gram of pepsin to a liter of N/20 HCl. The manipulation was the same as in experiment 7 up to the point of filtering. Instead of heating the contents of the beakers on the water bath for about 1/2 hour before filtering they were evaporated to 15 c. c. Then they were filtered and the filtrates caught in 110 c. c. graduated flasks. The residues

were washed thoroughly and the filtrates made up to the 110 c. c. mark. The residues were transferred to Kjeldahl flasks and the N determined as usual. Two fifty c. c. portions of the filtrate were then taken, one for nitrogen determination, and the other for the estimation of albumoses by the $ZnSO_4$ method. As the residues from filtration gave the undigested N they were lettered z. One 50 c. c. portion of the filtrate from each beaker gave the nitrogen digested so it was lettered x, and the other 50 c. c. portion gave the N in the albumoses so it was lettered y. Method for (b), (d), (e), (f), and (g). Same as for (a) except that different digestive fluids were used. Method for (h). Twelve samples of the meat were weighed out and a water extract made. The samples were kept separate and labelled from 2193/1 to 2193/12 inclusive. Four of the residues were then treated exactly as in (a). These were numbered 2193/1z to 2193/4z inclusive. The portions of the filtrates taken for the N determination were labelled 2193/1x tl 2193/4x and the portions used for the albumoses were marked 2193/1y to 2193/4y. Four more of the residues (Nos. 2193/5 to 2193/8 inclusive) were treated as in (b) and the different portions lettered as before. The remaining four (Nos. 2193/9 to 2193/12 inclusive) were treated as in (c).

The results of the entire experiment are given in tables 8 and 9.

Table 8.

Experiment 8.

Exp. No.	Lab. No.	Strength of Acid.	Pepsin.	Per cent of undigested nitrogen.	Per cent of digested nitrogen.	Per cent of total N found in albumoses.
8a	2186/1z	N/20	1/2 gm-l	6.49		
"	2186/2z	"	"	6.92		
"	2186/3z	"	"	5.87		
"	2186/4z	"	"	5.95		
"	2186/5z	"	"	6.80		
	Average----------			-6.41		
"	2186/1x	"	"		80.92#	
"	2186/2x	"	"		96.15	
"	2186/3x	"	"		96.16	
"	2186/4x	"	"		96.46	
"	2186/5x	"	"		48.27#	
	Average----------				-96.26	
"	2186/1y	"	"			64.87
"	2186/2y	"	"			58.12
"	2186/3y	"	"			60.48
"	2186/4y	"	"			58.44
"	2186/5y	"	"			58.29
	Average----------					-60.4
8b	2187/1z	"	None	74.52		
"	2187/2z	"	"	70.95		
"	2187/3z	"	"	68.22		
"	2187/4z	"	"	70.02		
"	2187/5z	"	"	75.53		
	Average----------			-71.85		
"	2187/1x	"	None		24.86	
"	2187/2x	"	"		27.85	
"	2187/3x	"	"		21.05	
"	2187/4x	"	"		-----	
"	2187/5x	"	"		22.22	
	Average----------				-24.00	

This number is not included in the averages.

Table 8.

Experiment 8.

Exp.	Lab. No.	Solution of	Per cent of	Per cent of	Per cent of
		Acetylsalicylic Acid.	Unadulterated	Glycerine.	Total Ash
			Solution.		

Table

Experiment 8.

Exp. No.	Lab. No.	Strength of Acid.	Pepsin.	Per cent of undigested nitrogen.	Per cent of digested nitrogen.	Per cent of total N found in albumoses.
8b	2187/1y	N/20	None			-----
"	2187/2y	"	"			16.07
"	2187/3y	"	"			11.72
"	2187/4y	"	"			-----
"	2187/5y	"	"			10.46
	Average					12.75
8c	2188/1z	None	None	83.64		
"	2188/2z	"	"	85.17		
"	2188/3z	"	"	-----		
"	2188/4z	"	"	82.96		
"	2188/5z	"	"	83.85		
	Average			83.90		
"	2188/1x	"	"		16.07	
"	2188/2x	"	"		16.31	
"	2188/3x	"	"		-----	
"	2188/4x	"	"		15.56	
"	2188/5x	"	"		16.51	
	Average				16.11	
"	2188/1y	"	"			5.10
"	2188/2y	"	"			5.01
"	2188/3y	"	"			-----
"	2188/4y	"	"			5.07
"	2188/5y	"	"			5.49
	Average					5.17
8d	2189/1z	"	1/2 gm-l	76.95		
"	2189/2z	"	"	76.89		
"	2189/3z	"	"	77.12		
"	2189/4z	"	"	-----		
"	2189/5z	"	"	77.30		
	Average			77.06		

Table

Experiments 8.

Exp. No.	Lab. No.	Strength of Acid.Formula.	Per cent of unoxidized nitrogen.	Per cent of nitrogen oxidized.	Per cent of Total N found in ammonia.
81	2187/1x	1/20	None		
	2187/2y				11.07
	2187/3y				13.73
	2187/4y				
	2187/5y				10.45
Average					13.75
82	2188/1x	None	None	53.44	
	2188/2x			85.17	
	2188/3x				
	2188/4x			83.94	
	2188/5x			83.05	
Average				83.90	
	2189/1x			16.07	
	2189/2x			16.31	
	2189/3x				
	2189/4x			16.54	
	2189/5x			16.51	
Average				16.11	
	2190/1y			5.10	
	2188/2y			5.01	
	2189/3y				
	2190/4y			5.07	
	2190/5y			5.49	
Average				5.17	
84	2187/1x	1/2 Sp-1		76.45	
	2190/2x			76.89	
	2189/3x			77.13	
	2189/4x				
	2187/5x			77.30	
Average				77.94	

Table 8.

Experiment 8.

Exp. No.	Lab. No.	Strength of Acid.	Pepsin.	Per cent of undigested nitrogen.	Per cent of digested nitrogen.	Per cent of total N found in albumoses.
8d	2189/1x	None	1/2 gm-l		27.34	
"	2189/2x	"	"		25.25	
"	2189/3x	"	"		24.60	
"	2189/4x	"	"		26.60	
"	2189/5x	"	"		23.20	
	Average				25.40	
"	2189/1y	"	"			11.17
"	2189/2y	"	"			9.02
"	2189/3y	"	"			----
"	2189/4y	"	"			----
"	2189/5y	"	"			7.52
	Average					7.24
8e	2190/1z	N/40	"	9.89		
"	2190/2z	"	"	9.35		
"	2190/3z	"	"	10.17		
"	2190/4z	"	"	7.39		
"	2190/5z	"	"			
	Average			9.20		
"	2190/1x	"	"		-----	
"	2190/2x	"	"		92.67	
"	2190/3x	"	"		-----	
"	2190/4x	"	"		95.62	
"	2190/5x	"	"		96.85	
	Average				95.05	
"	2190/1y	"	"			-----
"	2190/2y	"	"			52.57
"	2190/3y	"	"			51.69
"	2190/4y	"	"			55.56
"	2190/5y	"	"			-----
	Average					53.27

Table 5.

Experiment N.

Exp. No.	Lab. No.	Strength of Acid.Pepsin.	Per cent of undigested nitrogen.	Per cent nf digested nitrogen.	Per cent nf total N found in filtrate.
64	21307/1x	none 1/2 gm-1		37.34	
	21309/2x			85.85	
	21314/3x			94.50	
	21315/4x			95.00	
	21316/5x			95.00	
	Average		95.40		
	21321/1y				11.17
	21323/2y				9.05
	21324/3y				----
	21325/4y				----
	21326/5y				7.35
	Average				7.96
66	21330/1x	1/40	5.78		
	21331/2x				
	21332/3x				
	21333/4x				
	21334/5x				
	Average		1.30		
	21340/1x				
	21342/2x			59.87	
	21343/3x			----	
	21344/4x			95.43	
	21345/5x			96.45	
	Average		95.44		
	21352/1y				----
	21353/2y				32.37
	21354/3y				21.79
	21355/4y				13.35
	21356/5y				----
	Average				22.50

Table 8.

Experiment 8.

Exp. No.	Lab. No.	Strength of Acid.	Pepsin.	Per cent of undigested nitrogen.	Per cent of digested nitrogen.	Per cent of total N found in albumoses.
8f	2191/1z	N/100	1/2 gm-l	31.87		
"	2191/2z	"	"	25.68		
"	2191/3z	"	"	18.26		
"	2191/4z	"	"	17.48		
"	2191/5z	"	"	22.74		
	Average			23.21		
"	2191/1x	"	"		70.14	
"	2191/2x	"	"		75.48	
"	2191/3x	"	"		76.54	
"	2191/4x	"	"		84.78	
"	2191/5x	"	"		33.78#	
	Average				77.48	
"	2191/1y	"	"			-----
"	2191/2y	"	"			-----
"	2191/3y	"	"			42.94
"	2191/4y	"	"			45.79
"	2191/5y	"	"			42.47
	Average					43.73
8h	2193/1z	N/20	1/2 gm-l	6.77		
"	2193/2z	"	"	6.34		
"	2193/3z	"	"	5.66		
"	2193/4	"	"	6.62		
	Average			6.35		
"	2193/1x	"	"		76.82	
"	2193/2x	"	"		76.70	
"	2193/3x	"	"		75.28	
"	2193/4x	"	"		77.98	
	Average				76.69	

This number is not included in the numbers averaged.

Table 7.

Experiment 8.

Exp. No.	Lab. No.	Strength of Acid.Protein.	Per cent of unhydrated nitrogen.	Per cent of digested nitrogen.	Per cent of total N found in filtrate.
87	2191/1x	N/100 1/2 Sp-I	31.87		
"	2191/2x	"	25.58		
"	2191/3x	"	18.96		
"	2191/4x	"	17.68		
"	2191/5x	"	22.74		
	Average		23.37		
"	2191/1x	"		70.14	
"	2191/2x	"		72.48	
"	2191/3x	"		76.54	
"	2191/4x	"		84.72	
"	2191/5x	"		83.78	
	Average			77.68	
"	2191/1x	"			
"	2191/2x	"			
"	2191/3x	"			
"	2191/4x	"			42.74
"	2191/5x	"			43.72
	Average				43.23
88	2193/1x	N/20 1/2 Sp-I	6.77		
"	2193/2x	"	6.34		
"	2193/3x	"	5.64		
"	2193/4	"	6.82		
	Average		6.39		
"	2193/1x	"		74.82	
"	2193/2x	"		74.70	
"	2193/3x	"		75.34	
"	2193/4x	"		77.88	
	Average			75.69	

* This number is not included in the computed average.

Table 8.

Experiment 8.

Exp. No.	Lab. No.	Strength of Acid.	Pepsin.	Per cent of undigested nitrogen.	Per cent of digested nitrogen.	Per cent of total N found in albumoses.
8h	2193/1y	N/20	1/2 gm-l			48.46
"	2193/2y	"	"			45.66
"	2193/3y	"	"			42.47
"	2193/4y	"	"			45.54
	Average					45.53
"	2193/5z	N/100	"	-----		
"	2193/6z	"	"	-----		
"	2193/7z	"	"	-----		
"	2193/8z	"	"	53.04		
	Average			53.04		
"	2193/5x	"	"		-----	
"	2193/6x	"	"		21.25	
"	2193/7x	"	"		-----	
"	2193/8x	"	"		24.83	
	Average				23.04	
"	2193/5y	"	"			-----
"	2193/6y	"	"			19.38
"	2193/7y	"	"			------
"	2193/8y	"	"			22.34
	Average					20.86
"	2193/9z	"	"	72.28		
"	2193/10z	"	"	-----		
"	2193/11z	"	"	-----		
"	2193/12z	"	"	73.41		
	Average			72.84		
"	2193/9x	"	"		6.89	
"	2193/10x	"	"		----	
"	2193/11x	"	"		----	
"	2193/12x	"	"		7.51	
	Average				7.20	

Table 7.

Experiment 6.

Exp. No.	Lab. No.	Strength of Acid.	Per cents of Protein.	Per cents of Undigested	Per cents of Total N Found in Filtrate.

Table 8.

Experiment 8.

Exp. No.	Lab. No.	Strength of Acid.	Strength of Pepsin.	Per cent of undigested nitrogen.	Per cent of digested nitrogen.	Per cent of total N found in albumoses.
8h	2193/9y	N/100	1/2 gm-l			6.03
"	2193/10y	"	"			----
"	2193/11y	"	"			----
"	2193/12y	"	"			----
	Average					6.03

Table 6.

Experiment 6.

Exp. No.	Lab. No.	Strength of Acid.Per cent. undigested.	Per cent of digested nitrogen.	Per cent of total N found in filtrate.
				6.04
				6.08

Table 9.

Experiment 8. Summary.

Exp. No.	Lab. No.	Per cent of undigested N.	Per cent of digested N.	Digested and undigested N.	Per cent of total N as albumoses.	Digested N - albumoses = per cent N as peptones and end product.
8a	2186/1	6.49	80.92	87.41	64.87	16.05
"	2186/2	6.92	96.15	103.07	58.12	38.03
"	2186/3	5.87	96.16	102.03	60.48	35.68
"	2186/4	5.95	96.46	102/41	58.44	38.02
"	2186/5	6.80	48.27#	------	58.29	-----
Average		6.41	96.26	102.67	60.40	35.86
8b	2187/1	74.52	24.86	99.38	-----	-----
"	2187/2	70.95	27.85	98.80	16.07	11·78
"	2187/3	68.22	21.05	89.27	11.72	9.33
"	2187/4	70.02	-----	-----	-----	-----
"	2187/5	75.53	22.22	97.75	10.46	11.76
Average		71.85	24.oo	95.85	12.75	11.25
8c	2188/1	83.64	16.07	99.71	5.10	10.97
"	2188/2	85.17	16.31	101.48	5.01	11.30
"	2188/3	-----	-----	------	------	-----
"	2188/4	82.96	15.56	98.53	5.07	10.49
"	2188/5	83.85	16.51	100.36	5.49	11.02
Average		83.90	16.11	100.01	5.17	10.94
8d	2189/1	76.95	27.34	104.29	11.17	16.17
"	2189/2	76.89	25.25	102.14	9.02	16.23
"	2189/3	77.12	24.60	101.72	----	-----
"	2189/4	-----	26.60	------	----	-----
"	2189/5	77.30	23.20	100.50	7.52	15.68
Average		77.06	25.40	102.46	7.24	18.16
8e	2190/1	-----	-----	------	----	-----
"	2190/2	9.89	92.67	102.56	52.57	40.10
"	2190/3	9.35	-----	------	51.69	-----
"	219o14	10.17	95.62	105.79	55.56	40.06
"	2190/5	7.39	96.85	104.24	-----	-----
Average		9.20	95.05	104.25	53.27	41.78

This number is not included in the numbers averaged.

Table .

Experiment 6. Diameter.

Exp.	Lab.	Per cent	Per cent	Digested	Per cent	Digested N -
No.	No.	of	of	And	Of Total	Digestible
		Water	Dry	Nitro-	N as	Per cent N as
		Lost	N.	genous	Albu-	Digestible Alb
				N.	mosen.	umin Protein.

Table 9.

Experiment 8. Summary.

Exp. No.	Lab. No.	Per cent of undigested N.	Per cent of digested N.	Digested and undigested. N.	Per cent of total N as albumoses.	Digested N - albumoses = per cent N as peptones and end products.
8f	2191/1	31.87	70.14	102.01	-----	-----
"	2191/2	25.68	75.48	101.16	-----	-----
"	2191/3	18.26	76.54	94.80	42.94	33.60
"	2191/4	17.48	84.78	102.26	45.79	38.99
"	2191/5	22.74	33.78# 76.74		42.47	-----
Average------		23.21		100.69	43.73	33.01
8h	2193/1	6.77	76.82	83.59	48.46	28.36
"	2193/2	6.34	76.70	83.04	45.66	31.04
"	2193/3	5.66	75.28	80.94	42.47	32.88
"	2193/4	6.62	77.98	84.60	45.54	33.44
Average-------		6.35	76.69	83.04	45.53	31.16
"	2193/5	----	-----	-----	-----	-----
"	2193/6	----	21.25	-----	19.38	1.87
"	2193/7	----	-----	-----	-----	----
"	2193/8	53.04	24.83	77.87	22.34	2.49
Average------		53.04	23.04	76.08	20.86	2.18
"	2193/9	72.28	6.89	79.17	6.03	.86
"	2193/10	-----	----	-----	----	---
"	2193/11	-----	----	-----	----	---
"	2193/12	73.41	7.51	80.92	----	---
Average------		72.84	7.20	80.04	6.03	1.17

This number is not included in the numbers averaged.

Observations and Conclusions. From the summary, table 9, it is evident that in (a), when an acid pepsin solution containing 1 gram of pepsin to a liter of N/20 HCl was used, about 96% of the meat was digested in one hour. Sixty per cent had been converted into albumoses, losing about 36% as peptones and further end products. The sum of the digested and the undigested N was 102.67%. Although there was considerable variation in duplicates, in some cases these figures probably represent approximately what was present.

In (b), where the acid was used alone, only about 24 per cent was digested, 13 per cent being albumoses and 11 per cent further end products. The sum of the digested and undigested portions averaged about 96 per cent.

In (c) when water alone was used, the digestible portion was 16 per cent and the undigested 84 per cent. Five per cent was found as albumoses and 11 per cent as further end products. A comparison of (b) and (c) is interesting in determining the effect of the HCl alone on the digestive action. It is clear that the difference between 24 per cent and 16 per cent is 8 per cent and represents approximately the amount of digestion produced by the HCl itself. Further it is to be noticed that the difference between the albumoses in (b) and those in (c) is also about 8 per cent. It is reasonable to assume therefore, that the HCl digested about 8 per cent of the

meat more than the water alone and all of this increase was found as albumoses.

The results from (d) show that when pepsin alone is used (1 gm-l), only about 25 per cent of the sample is digested. This is practically the same amount digested by the N/20 acid alone. In this case however a larger per cent remains undigested than when the acid alone was used, the amount of albumoses produced is smaller, and the peptones and further end products increased. It seems then that the pepsin of this strength has no greater digestive power than the N/20 acid alone but that a large per cent of the digested portion is carried beyond the albumose stage.

When the acidity of the acid pepsin solution used in (a) is decreased to N/40 as in (e), the amount digested is about the same as with the stronger acid. This is in harmony with the previous results of this study. It seems however that in this case the quantity of albumoses produced is less than with the stronger acid, and the amount of peptones and further end products, greater. This indicates that the weaker acid allows the pepsin to work to better advantage than the stronger acid.

In (f), the strength of the acid was decreased to N/100. A decided decrease in digestibility resulted although the soluble portion was still high. This shows that an extremely

weak acid is necessary to retard the action to an appreciable extent, a conclusion that our former results tend to support. The proportion of albumoses and peptones present after digestion in (f), are about the same as in (e).

No results from section (g) of this experiment were obtained so this letter was omitted from the table.

From (h) we find that approximately 80 per cent of the meat sample remained after extracting with water. The water extract therefore must have amounted to about 20 per cent. Of the 80 per cent remaining, 76 per cent was digested with the acid pepsin solution containing 1 gram of pepsin to a liter of N/20 acid, 23 per cent with the acid alone, and 7 per cent with water alone. The proportion therefore is 76 : 23 : 7. When the fresh meat was digested in (a), (b), and (c) the proportion under the same conditions was 96 : 24 : 16. The advantage in the former proportion is first, in favor of the acid alone, next the acid pepsin, and lastly the water. The acid alone digested as much of the residue from the water extract as the fresh meat, the acid pepsin as much of the residue as the fresh meat minus the water extract, and the water alone only about 1/2 as much as with the fresh meat. Further, when the acid pepsin was used, more than half of the digested portion was found as albumose. This is similar to the action on the fresh meat.

When acid alone was used nearly all of the digested portion was found as albumoses. This also agrees with the results with fresh meat. When water alone was used, a little larger per cent was found as albumoses than with the fresh meat.

On the whole the results of experiment 8 indicate many interesting things, some of them expected and some unexpected. It is not intended however to draw definite conclusions from the limited number of facts here shown. More results will have to be obtained. Furthermore the method used here is not sufficiently developed for the most accurate work.

Experiment 9.

Object. To determine the rate of digestion in a pepsin solution containing 1/2 gram of pepsin to a liter of water, when the solution is made weakly acid at the end of the first hour.

Method. Twenty five samples of meat of from 2-2.5 grams each were weighed out into 25 c. c. Jena beakers and labelled from 2195/1 to 2195/25 inclusive. Five c. c. of water was added and the contents stirred one minute. To each beaker 100 c. c. H_2O were added containing 1/2 gram pepsin per liter. They were all digested at 40° C. for one hour. At the end of one hour five samples (2195/1 to 2195/5 inclusive- were removed,

Table 10.

Rate of digestion of a sample of beef.

Experiment 9.

Exp. No.	Lab. No.	Time of digestion.	C. c. of N/10 HCl at end of first hour.	Per cent of undigested N.	Per cent of digested N.
9	2195/1z	1 hr.	None	77.70	
"	2195/2z	"	"	76.79	
"	2195/3z	"	"	76.58	
"	2195/4z	"	"	75.48	
"	2195/5z	"	"	-----	
	Average			76.64	
"	2195/1x	"	"		26.04
"	2195/2x	"	"		26.93
"	2195/3x	"	"		26.70
"	2195/4x	"	"		28.21
	Average				26.97
"	2195/5x	"	"		24.54
"	2195/6z	2 hrs.	2 c. c.	64.81	
"	2195/7z	"	"	70.35	
"	2195/8z	"	"	64.31	
"	2195/9z	"	"	68.09	
	Average			66.89	
"	2195/10z	"	"	-----	
"	2195/6x	"	"		38.78
"	2195/7x	"	"		32.57
"	2195/8x	"	"		38.68
"	2195/9x	"	"		-----
	Average				36.67
"	2195/10x	"	"		16.80#
"	2195/11z	3 hrs.	"	62.89	
"	2195/12z	"	"	62.78	
"	2195/13z	"	"	62.53	
"	2195/14z	"	"	64.97	
	Average			63.29	
"	2195/15z	"	"	-----	

This number isnot included in the numbbers averaged.

Table 10.

Rate of digestion of a sample of beef.

Experiment 9.

Exp. No.	Lab. No.	Time of digestion.	C. c. of N/10 HCl at end of first hour.	Per cent of undigested N.	Per cent of digested N.
9	2195/11x	3 hrs.	2 c. c.		44.05
"	2195/12x	"	"		41.30
"	2195/13x	"	"		40.31
"	2195/14x	"	"		39.50
	Average				31.29
"	2195/15x	"	"		36.41
"	2195/16z	4 hrs.	"	-----	
"	2195/17z	"	"	62.12	
"	2195/18z	"	"	60.35	
"	2195/19z	"	"	59.16	
	Average			60.54	
"	2195/20z	"	"	-----	
"	2195/16x	"	"		40.89
"	2195/17x	"	"		41.25
"	2195/18x	"	"		42.63
"	2195/19x	"	"		45.14
	Average				42.48
"	2195/20x	"	"		47.01
"	2195/21z	5 hrs.	"	60.51	
"	2195/22z	"	"	55.15	
"	2195/23z	"	"	53.56	
"	2195/24z	"	"	49.62	
	Average			54.71	
"	2195/25z	"	"	-----	
"	2195/21x	"	"		41.32
"	2195/22x	"	"		47.79
"	2195/23x	"	"		50.82
"	2195/24x	"	"		53.31
	Average				48.31
"	2195/25x	"	"		-----

neutralized with 2 c. c. $BaCO_3$ paste, and evaporated to 15 c. c. on the water bath. When the first five beakers were removed 2 c. c. of N/10 HCl were added to the contents of each of the beakers remaining in the digesting bath. At the end of the second hour, five more samples were removed, neutralized, and evaporated. These were Nos. 2195/6 to 2195/10. At the end of the third hour five more samples were removed, neutralized, and filtered (Nos. 2195/11 to 2195/15). This process was repeated at the end of each succeeding hour until all the beakers were removed.

When the contents of all the beakers were evaporated to 15 c. c., four from each lot of five were filtered into 110 c. c. measuring flasks and the residues washed thoroughly. The N was determined in the residues and in 50 c. c. portions of the filtrates. The last sample from each lot of five was diluted to 250 c. c., stirred, and filtered through a dry filter into a 200 c. c. measuring flask. The residues were not washed. The N was determined in the residues and in 200 c. c. portions of the filtrate.

In all cases the residues were lettered z and the filtrates x with the proper lab. no. The table of results follows table No. 10.

Observations and conclusions. In this experiment we get for the first time results which show the rate of digestion when the amount digested during the initial period

is small. The acidity of the solution is exceedingly small, being approximately N/500 after the first hour. In the table the results of the last sample of each lot of five was not averaged in with the others because it was treated in a little different manner as mentioned above. It is given below the average in each case, and does not show much uniformity with it in most cases.

By examining the tables we see that approximately 26 per cent of the meat is digested in one hour by the pepsin solution alone. This agrees very well with the resultsof experiment 8 (d). By making the solution N/500 acid, the digestion increases gradually until at the end of five hours it has reached nearly 50 per cent. It is evident therefore that in experimenting upon the rate of digestion of beef a very weak acidity is desirable.

Experiment 10.

Object. To determine the rate of digestion of beef in an acid pepsin solution in which the acidity was increased by a definite amount at regular intervals.

Method. The experiment was divided into four parts, a, b, c, and d.

(a) Five portions of thoroughly mixed lean beef

of from 2 to 2.5 grams each were weighed out into Jena beakers of 250 c. c. capacity and labelled 2190/1 to 2190/5 inclusive. Five c. c. NH_3 free H_2O were added and the contents stirred to a thick paste with a glass rod. To each beaker 100 c. c. H_2O containing 1/2 gram pepsin per liter were added. The mixture was digested at 40° for 1 hour. At the end of 1 hour 2 c. c. of N/10 HCl were added. At the end of 2 hours digestion 2 c. c. more of N/10 HCl were added and this process was repeated at end of each succeeding hour until 8 c. c. N/10 HCl had been added. Digestion was continued 1 hour longer and the solution was then neutralized with 2 c. c. $BaCO_3$ paste. After evaporating to small volume it was filtered and the residue thoroughly washed with hot water. The N was determined in the residue and also in a 50 c. c. portion of the filtrate after it had been made up to 110 c. c. The residues were labelled z and the filtrates x.

(b) Same as above except that only six c. c. N/10 HCl were added in portions of 2 c. c. each. Therefore the period of digestion was cut from 6 to 5 hours. The laboratory numbersincluded 2196/6 and 2196/10.

(c) Same as above except that only 4 c. c. N/10 HCl were added. Digestion was cut from 5 to 4 hours. The laboratory numbers included 2196/11 to 2196/15.

(d) Same as in (c) except that no acid was added, and the

Table 11.

Rate of digestion with variable acidity.

Experiment 10.

Exp. No.	Lab. No.	Time of digestion.	C. c. of N/10 HCl added in portions of 2 c.c. each, at regular intervals.	Per cent of undigested N.	Per cent of digested N.
10a	2196/1z	5 hrs.	8 c. c.	50.19	
"	2196/2z	"	"	48.41	
"	2196/3z	"	"	38.16	
"	2196/4z	"	"	45.24	
"	2196/5z	"	"	52.38	
	Average			47.07	
"	2196/1x	"	"		53.16
"	2196/2x	"	"		57.33
"	2196/3x	"	"		65.35
"	2196/4x	"	"		58.35
"	2196/5x	"	"		51.16
	Average				57.07
10b	2196/6z	4 hrs.	6 c. c.	54.29	
"	2196/7z	"	"	46.69	
"	2196/8z	"	"	53.84	
"	2196/9z	"	"	56.79	
"	2196/10z	"	"	51.04	
	Average			52.53	
"	2196/6x	"	"		49.50
"	2196/7x	"	"		57.44
"	2196/8x	"	"		49.09
"	2196/9x	"	"		-----
"	2196/10x	"	"		52.22
	Average				52.06

Table 11.

Rate of digestion with variable acidity.

Experiment 10.

Exp. No.	Lab. No.	Time of digestion.	C. c. of N/10 HCl added in portions of 2 c. c. each, at regular intervals.	Per cent of undigested N.	Per cent of digested N.
10c	2196/11z	3 hrs.	4 c. c.	56.22	
"	2196/12z	"	"	50.71	
"	2196/13z	"	"	53.21	
"	2196/14z	"	"	60.08	
"	2196/15z	"	"	54.72	
	Average			54.99	
"	2196/11x	"	"		50.05
"	2196/12x	"	"		54.61
"	2196/13x	"	"		51.30
"	2196/14x	"	"		44.66
"	2196/15x	"	"		47.63
	Average				49.65
10d	2196/16z	28 hrs.	None	69.36	
"	2196/17z	"	"	66.03	
"	2196/18z	"	"	65.61	
"	2196/19z	"	"	-----	
"	2196/20z	"	"	63.36	
	Average			66.09	
"	2196/16x	"	"		34.59
"	2196/17x	"	"		-----
"	2196/18x	"	"		37.80
"	2196/19x	"	"		33.61
"	2196/20x	"	"		36.63
	Average				35.66

digestion was continued for 21 hours,-- 13 hours at 40° and 15 at the temperature of the laboratory.

Observations and conclusions. From (d) we see that the pepsin without the acid digested 35 per cent of the meat in 28 hours, or 10 per cent more than it did in the two previous experiments for one hour. When the acid was added at regular intervals the digestion increased steadily from 49 per cent in three hours to 57 per cent in 5 hours. This took place in solutions which varied in acidity from N/500 to N/125.

The same irregularities in duplicates appears in this series as in previous experiments in spite of the fact that great care was exercised in manipulation.

Experiment 11.

Object. To determine the digestibility of beef in weak acid solution when the acidity is increased by a constant amount at regular intervals.

Method. The experiment was divided into four parts, a, b, c, and d and each part carried out exactly as in the preceding experiment, except that the pepsin was ommitted from the digesting solution. The results are given in table 12.

Observations and Conclusions. The per cent digested in a, b, and c, is practically constant, showing that when the

Table 12.

Rate of digestion in acid alone with variable acidity.

Experiment 11.

Exp. No.	Lab. No.	Time of digestion.	C. c. of N/10 HCl added in portions of 2 c.c. each at regular intervals.	Per cent of undigested N.	Per cent of digested N.
11a	2197/1z	5 hrs.	8 c. c.	83.81	
"	2197/2z	"	"	84.65	
"	2197/3z	"	"	84.21	
"	2197/4z	"	"	84.69	
"	2197/5z	"	"	85.18	
	Average			84.51	
"	2197/1x	"	"		16.00
"	2197/2x	"	"		16.03
"	2197/3x	"	"		30.05#
"	2197/4x	"	"		16.06
"	2197/5x	"	"		16.65
	Average				16.19
11b	2197/6z	4 hrs.	6 c. c.	83.60	
"	2197/7z	"	"	84.85	
"	2197/8z	"	"	84.33	
"	2197/9z	"	"	85.41	
"	2197/10z	"	"	84.28	
	Average			84.49	
"	2197/6x	"	"		15.87
"	2197/7x	"	"		15.95
"	2197/8x	"	"		15.82
"	2197/9x	"	"		-----
"	2197/10x	"	"		13.31
	Average				15.27

This number is not included in the numbers averaged.

Table 12.

Rate of digestion in acid alone with variable acidity.

Experiment 11.

Exp. No.	Lab. No.	Time of digestion.	C. c. of N/10 HCl added in portions of 2 c. c. each at regular intervals.	Per cent of undigested N.	Per cent of digested N.
11c	2197/11z	3 hrs.	4 c.c.	84.39	
"	2197/12z	"	"	82.56	
"	2197/13	"	"	82.62	
"	2197/14z	"	"	90.11	
"	2197/15z	"	"	85.18	
	Average----------			-84.97	
"	2197/11x	"	"		14.88
"	2197/12x	"	"		15.70
"	2197/13	"	"		14.86
"	2197/14	"	"		15.25
"	2197/15x	"	"		15.29
	Average----------				-15.20

acid solution is between N/500 and N/125 at all times the periodic increases in acidity do not cause an increase in digestion. This fact taken in connection with the results of Experiment 10 indicates that an increase of acidity from N/500 to N/125 causes an increase in digestibility only when the pepsin is present. As the pepsin without the acid has little power of digestion of its own, they must both be present.

Experiment 12.

Since the last four experiments had been carried on with great care, and the desired uniformity in duplicates had not been obtained, it was decided that something besides faulty neutralization was the cause of the variation. The only other explanation of the difficulty was that the sample was not sufficiently fine for good peptonization. Experiment 12 was therefore planned with the following object.

Object. To compare the ordinary method of manipulation with one in which the meat was ground with some hard granular substance. The purpose of such treatment was to keep the meat particles separated as completely as possible during digestion.

Method. Fifteen samples of meat were weighed out as usual, and numbered from 2198/1 to 2198/15 inclusive. The first five samples were treated with 5 c. c. of H_2O and stirred with a

Table 13.

Comparison of method of getting sample finely divided.

Experiment 12.

Exp. No.	Lab. No.	Time of digestion.	C. c. of N/10 HCl added in portions of 2 c. c. each at regular intervals.	Substance ground with meat.	Per cent undigested.
12	2198/1	3 hrs.	4 c. c.	None	52.05
"	2198/2	"	"	"	52.49
"	2198/3	"	"	"	-----
"	2198/4	"	"	"	51.75
"	2198/5	"	"	"	52.09
	Average				52.10
"	2198/6	"	"	Silica	50.38
"	2198/7	"	"	"	57.38
"	2198/8	"	"	"	52.01
"	2198/9	"	"	"	44.10
"	2198/10	"	"	"	59.53
	Average				52.68
"	2198/11	"	"	Pumice	46.96
"	2198/12	"	"	"	55.63
"	2198/13	"	"	"	57.18
"	2198/14	"	"	"	52.97
"	2198/15	"	"	"	53.94
	Average				53.34

glass rod until a thick paste had formed, as usual. One hundred c. c. of water containing 1/2 gram of pepsin per liter were then added and the samples digested for three hours. At the end of the first hour 2 c. c. of N/10 HCl were added and at the end of the second hour, this was repeated. Neutalization and filtration were accomplished as usual. The N was determined in the residues but not in the filtrates. The second five samples were treated exactly as the first except that a little precipitated silica was ground into the meat as thoroughly as possible with a glass rod after the first 5 c. c. of water wereadded. The third set of five were treated exactly as the second set with pumice substituted for silica. The results of the experiment are given in table 13.

Observations and Conclusions. Since the purpose of the experiment was to determine which of the three treatments gave the most uniform results, a glance at the table shows that neither silica nor pumice further the desired end. In fact the results in the first set are much better than those in the other two. Another method therefore had to be tried for dividing the sample more finely.

Experiment 13.

Object. To eliminate the variations in duplicates by

Table 14.

Digestion results when meat was ground as finely as possible with glass rod.

Experiment 13.

Exp. No.	Lab. No.	Time of digestion.	C. c. of N/10 HCl added in portions of 2 c. c. each at regular intervals.	Per cent of undigested N.	Per cent of digested N.
13	2208/1z	3 hrs.	4 c. c.	57.31	
"	2208/2z	"	"	53.31	
"	2208/3z	"	"	57.06	
"	2208/4z	"	"	52.65	
"	2208/5z	"	"	60.05	
	Average			56.07	
"	2208/1x	"	"		44.27
"	2208/2x	"	"		48.79
"	2208/3x	"	"		45.55
"	2208/4x	"	"		-----
"	2208/5x	"	"		43.12
	Average				45.43
"	2208/6z	"	"	42.14#	
"	2208/7z	"	"	57.85	
"	2208/8z	"	"	-----	
"	2208/9z	"	"	-----	
"	2208/10z	"	"	53.09	
	Average			55.47	
"	2208/6x	"	"		44.47
"	2208/7x	"	"		44.95
"	2208/8x	"	"		45.93
"	2208/9x	"	"		-----
"	2208/10x	"	"		41.54
	Average				44.22

This number is not included in the numbers averaged.

dividing the meat as finely as possible with a glass rod flattened on the end.

Method. Ten samples of lean beef were weighed out as usual and treated with five c. c. of water. The meat was then ground as fine as possible with the flattened end of a glass rod. It was stirred and worked until no further division of the particles could be made in this manner. The process of digestion was then continued exactly as in experiment 12. After filtering the N was determined in both the residues and filtrates. The results are shown in table 14.

Observations and Conclusions. There is more variation in duplicates here than in many previous experiments.

Experiment 14.

One more attempt was made to determine whether the coarseness of the sample was responsible for the unsatisfactory variations in results. As it was thought the presence of connective tissue in the meat might be the cause of the trouble this experiment was planned with the following--

Object. To remove as much connective tissue as possible from the meat and compare the digestion results with those from samples which contained the connective tissue.

Method. A rather large portion of meat from sample

Table 15.

Comparative values foe meat ground through 40 mesh sieve and that not ground.

Experiment 14.

Exp. No.	Lab. No.	Condition of meat.	Time of digestion.	C. c. of N/10 HCl added in portions of 2 c. c. each at regular intervals.	Per cent not digested.	Per cent digested.
14	2212/1z	Ground.	2 1/2 hrs.	4 c. c.	-----	
"	2212/2z	"	"	"	51.41	
"	2212/3z	"	"	"	62.96	
"	2212/4z	"	"	"	60.92	
"	2212/5z	"	"	"	62.02	
	Average				59.33	
"	2212/1x	"	"	"		-----
"	2212/2x	"	"	"		52.31
"	2212/3x	"	"	"		39.23
"	2212/4x	"	"	"		41.41
"	2212/5x	"	"	"		34.87
	Average					41.96
"	2212/6z	Not ground	"	"	50.30	
"	2212/7z	"	"	"	-----	
"	2212/8z	"	"	"	45.63	
"	2212/9z	"	"	"	52.64	
"	2212/10z	"	"	"	51.57	
	Average				50.28	
"	2212/6x	"	"	"		51.62
"	2212/7x	"	"	"		-----
"	2212/8x	"	"	"		46.42
"	2212/9x	"	"	"		49.72
"	2212/10x	"	"	"		50.34
	Average					49.53

labelled 2212 was placed in a forty mesh sieve and rubbed with a pestel until sufficient meat had passed through the sieve to get five samples for digestion. The meat thus obtained was very finely divided and contained little or no connective tissue. When the samples had been weighed out they were labelled from 2212/1 to 2212/5 inclusive and digested for 2 1/2 hours in 100 c. c. of water containing 1/2 gram of pepsin per liter. At the end of the first hour 2 c. c. of N/10 HCl were added and at the end of the second hour this was repeated. After neutralizing and filtering the N was determined in both the residues and filtrates. Five more samples were obtained from the meat in the original condition (before grinding through sieve). Digestion was carried on as with the first five. The laboratory numbers for this set were from 2212/6 to 2212/10 inclusive.

Observations and Conclusions. There was less uniformity in the results with the ground meat than with the unground. From this data and that obtained in experiment 12 and 13, it was decided that dividing the sample more finely than that ordinarily used will not remedy the difficulty.

Experiment 15.

Since no remedy for the frequent non-uniformity of duplicates had been discovered when raw meat was digested, the

Table 16.

Digestion of pure beef proteid.

Experiment 15.

Exp. No.	Lab. No.	Time of digestion.	C. c. of N/10 HCl added in portions of 2 c. c. each at regular intervals.	Per cent of undigested. N.	Per cent of digested N. 100-z.
15	2236/1z	2 1/2 hrs.	6 c. c.	89.46	
"	2236/2z	"	"	-----	
"	2236/3z	"	"	88.62	
	Average			89.04	
"	2236/1x	"	"		10.54
"	2236/2x	"	"		-----
"	2236/3x	"	"		11.38
	Average				10.96
"	2236/4z	"	"	76.04	
"	2236/5z	"	"	-----	
"	2236/6z	"	"	75.94	
	Average			75.99	
"	2236/4x	"	"		23.96
"	2236/5x	"	"		-----
"	2236/6x	"	"		24.06
	Average				24.01

last experiment was carried on with a sample of pure beef proteid. The sample was obtained from Dr. P. F. Trowbridge.

Object. To determine whether the variations in the results would appear after digesting pure beef proteid, the same as with raw beef.

Method. Six samples of well dried proteid were weighed out and labelled from 2236/1 to 2236/6 inclusive. They were digested for 2 /12 hours in water containing 1/2 gram per liter and at the end of the first hour 2 c. c. N/10 HCl were added. This was repeated at the end of each succeeding half hour until 6 c. c. had been added. When the digestion was completed three samples (No. 2236/1 to 2236/3) were neutralized, evaporated, and filtered as usual. The other three (Nos. 2236/4 to 2236/6 inclusive) were heated on the water bath for half an hour and filtered without neutralization. The residues in all cases were lettered z and the filtrates x. The nitrogen was determined in each and the results given in table 16.

Observations and Conclusions. The per cent proteid digested in each case was small because the proteid is not so easily digested as the meat. All the duplicates however are fairly close, indicating that the difficulties encountered with the meat might be caused by factors in the meat which are not proteid in nature.

In this experiment a comparison is also made between the

method used in the beginning of the present study and that adopted later. In the latter method the solutions were neutralixed after digestion and evaporated to small volume before filtering. The difference between the amounts digested in the first and second cases is approximately 14 per cent. This must represent the per cent of acid albumin present in the solution after digestion.

CONCLUSIONS.

The conclusions to be drawn from the fifteen experiments of this study are of two kinds-- those relating to the method of procedure during artificial digestion, and those which refer to the qualitative and quantitative results of such digestion.

They may be tabulated as follows.

1. The method used in the first two experiments is impracticable for two reasons. 1st. The duplicates do not check and 2nd the filtration is too slow.

2. Formalin and phenol are both good antiseptics but formalin is preferred for this work when necessary.

3. Neutralizing the solution after digestion eliminates the difficulties of filtration. It does not make the filtrates perfectly clear however and the duplicates do not check as well as they should.

4. Evaporating on the water bath after neutralization to small volume increases speed of filtration and clearness of filtrates.

5. When KOH is used as the neutralizing agent and litmus paper for the indicator, it is impossible to obtain exactly similar neutral points in the different samples.

6. $BaCO_3$ is a better neutralizing agent

than KOH.

7. The coarseness of the sample, as used in the first experiments, is not the cause of the variation in the results.

8. Variations in results must be explained in one of two ways-- either the neutralization is not sufficiently accurate to precipitate all of the acid albumin in each case, or there is something in the meat besides the proteid bodies which affects the digestion. The evidences that the first explanation is correct are these: 1. After neutralization there was nearly always more or less turbidity in the filtrates. Some of the acid albumin therefore probably filtered through. 2. When the pure beef proteid was digested the duplicates by the neutralization method were not as good as by the method in which the solutions were not neutralized. The reasons for believing that this is not the cause of the variation in the duplicates, are as follows: 1. The greatest care was taken in the neutralization process to get the conditions the same in the different samples. 2. Only a small per cent of acid albumin is present in solution at any one time when beef is digested, and as careful neutralization must precipitate nearly all of this,there is little chance for error at this point. 3. When $BaCO_3$ paste is used the filtrates are almost perfectly clear, but the results are not always uniform. 4. When digestion

takes place with acid above the results are always more uniform than when acid pepsin solution is used. This is in spite of the fact that a greater amount of acid albumin is produced, which would tend to make the re ults more variable. The preponderance of evidence therefore is in favor of the second explanation offered above; namely, that there is something in the meat not of proteid nature, which causes the difficulty.

9. The digestive solution best fitted for determining is weakly acid-- between N/500 and N/100.

10. The products of digestion of meat in acid alone are acid albumin and a small amount of albumoses.

11. When the digestibility of a sample of meat is calculated from the amount of N found in the residues from filtration, practically the same results are obtained as when it is calculated from the N found in the filtrates. The sum of the N in the residues and the filtrates is nearly always very close to the total N of the meat.

12. The products of digestion of meat in a pepsin solution, without any acid, are, albumoses, peptones, and further end products. The albumoses seem to be converted into peptones almost as rapidly as they are formed.

13. When both the acid and pepsin are used the action

is much more rapid than with either one alone. The action of the pepsin however is hindered by a high acidity like N/20. It was found upon reducing the acidity to N/40 that a larger per cent of albumoses were converted into peptones than before.

14. The per cent of albumoses and further end products produced when the pepsin is constant varies with the strength of acid. Results from experiment 8 show that with a digestive fluid containing 1 gram of pepsin solution, the following relations existed:-- In one hour N/20 HCl produced 60 per cent albumoses and 35 per cent further end products. N/40 HCl produced 53 per cent albumoses and 41 per cent further end products. N/100 HCl produced 43 per cent albumoses and 41 per cent further end products.

In closing I wish to express my thanks to Dr. H. S. Grindley and Miss Marion E. Sparks for their assistance in connection with this work.

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OUTLINE OF CONTENTS.

A

B

Experiment 4. Rate of digestion of different wholesale cuts of beef.

Experiment 5. Rate of digestion of beef with acid pepsin solution containing 1 gram of pepsin to a liter of 0.2 per cent HCl, when initial period was reduced to 1/2 hour.

Experiment 6. Comparison of KOH and $BaCO_3$ as a neutralizing agent.

Experiment 7. Reduction of initial period of digestion to 10 min. and further tests of $BaCO_3$ as a neutralizing agent.

Conclusions from results obtained thus far, in regard to the method of conducting the experiments.

Experiment 8. Effect of the variation of strength of acid pepsin solution, the time of digesting being kept constant. Determination of albumoses produced during digestion.

Experiment 9. Rate of digestion of beef in water containing 1/2 gram of pepsin per liter when the solution is made weakly acid after the first hour.

Experiment 10. Rate of digestion of beef in acid pepsin solution when the acidity was increased by a definite amount at regular intervals.

Experiment 11. Rate of digestion of beef in a weak acid solution when the acidity is increased by a constant amount at regular intervals.

Experiment 12. Effect of silica and pumice as a means of getting beef sample divided for digestion.

Experiment 13. Effect of grinding sample as finely as possible with glass rod.

Experiment 14. Effect of grinding the beef through a forty mesh sieve before digestion.

Experiment 15. Digestion of pure beef proteid.

Conclusions drawn from experimental work.